September 30, 2021

OpGen, Inc.
% Randy Prebula
Partner; Hogan Lovells
Hogan Lovells, US LLP
Columbia Square
555 Thirteenth Street, NW
Washington, District of Columbia 20004

Re: K191288
Trade/Device Name: Acuitas AMR Gene Panel
Regulation Number: 21 CFR 866.1640
Regulation Name: Antimicrobial Susceptibility Test Powder
Regulatory Class: Class II
Product Code: PMY, OOI
Dated: October 13, 2020
Received: October 13, 2020

Dear Randy Prebula:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. Although this letter refers to your product as a device, please be aware that some cleared products may instead be combination products. The 510(k) Premarket Notification Database located at https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn.cfm identifies combination product submissions. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal

statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part 801 and Part 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803) for devices or postmarketing safety reporting (21 CFR 4, Subpart B) for combination products (see https://www.fda.gov/combination-products/guidance-regulatory-information/postmarketing-safety-reporting-combination-products); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820) for devices or current good manufacturing practices (21 CFR 4, Subpart A) for combination products; and, if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to https://www.fda.gov/medical-devices/medical-device-safety/medical-device-reporting-mdr-how-report-medical-device-problems.

For comprehensive regulatory information about medical devices and radiation-emitting products, including information about labeling regulations, please see Device Advice (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance) and CDRH Learn (https://www.fda.gov/training-and-continuing-education/cdrh-learn). Additionally, you may contact the Division of Industry and Consumer Education (DICE) to ask a question about a specific regulatory topic. See the DICE website (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance/contact-us-division-industry-and-consumer-education-dice) for more information or contact DICE by email (DICE@fda.hhs.gov) or phone (1-800-638-2041 or 301-796-7100).

Sincerely,

Ribhi Shawar, Ph.D. (ABMM)
Chief
General Bacteriology and Antimicrobial Susceptibility Branch
Division of Microbiology Devices
OHT7: Office of In Vitro Diagnostics and Radiological Health
Office of Product Evaluation and Quality
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: 06/30/2023
See PRA Statement below.

510(k) Number *(if known)*
K191288

Device Name
Acuitas AMR Gene Panel

Indications for Use *(Describe)*
The Acuitas® AMR Gene Panel, performed on the QIAGEN® EZ1® Advanced XL System and the OpGen Qualified QuantStudio™ 5 Real-Time PCR System, is a qualitative nucleic acid-based multiplex in vitro diagnostic test for detection and differentiation of antibiotic resistance markers to one or more antimicrobial agents. The test utilizes real-time polymerase chain reaction (PCR) and is performed on isolated colonies of Pseudomonas aeruginosa, Enterococcus faecalis, or members of Enterobacterales grown in pure culture on blood agar or MacConkey agar.

Organism identification results must be available prior to reporting results for the Acuitas AMR Gene Panel. Antimicrobial resistance gene results are reported by the Acuitas AMR Gene Panel for the combinations of bacterial pathogens and associated genetic resistance markers indicated in Table 1 below.

[continued on page 2]

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

[*continued from Form FDA 3881, page 1*]

Table 1 - Antimicrobial Resistance Gene Markers (Genetic Determinants) Associated with Bacterial Species

Organism	Reported AMR Gene Marker
Citrobacter freundii complex [a]	CTX-M-1, CTX-M-9, KPC, NDM, OXA-48
Citrobacter koseri	KPC, OXA-48
Enterobacter cloacae complex [b]	CTX-M-1, CTX-M-9, KPC, TEM [d]
Enterococcus faecalis	vanA
Escherichia coli	AAC, ANT, CMY, CTX-M-1, CTX-M-2, CTX-M-9, DFR, *gyr*A Mutant [c], KPC, MCR-1 [e], OXA-1, OXA-9, SHV [d], Sul1, Sul2, TEM [d]
Klebsiella aerogenes	CTX-M-1, CTX-M-9, KPC, NDM, OXA-48
Klebsiella michiganensis	CTX-M-1, CTX-M-9, KPC, NDM, OXA-48
Klebsiella oxytoca	CTX-M-1, CTX-M-9, KPC, NDM, OXA-48
Klebsiella pneumoniae	AAC, AAD, APH, CMY, CTX-M-1, CTX-M- 9, DFR, DHA, IMP, KPC, NDM, OXA-1, OXA-9, OXA-48, RMT, Sul1, Sul2, TEM [d]
Klebsiella quasipneumoniae	CTX-M-1, CTX-M-9, KPC, NDM, OXA-48
Klebsiella variicola	CTX-M-1, CTX-M-9, KPC, NDM, OXA-48
Morganella morganii	CTX-M-1, KPC, NDM, OXA-48
Proteus mirabilis	AAC, ANT, APH, armA, CMY, CTX-M-1, CTX-M-2, CTX-M-9, DFR, KPC, NDM, OXA-1, OXA-9, OXA-48, Sul2, TEM [d], VEB, VIM
Providencia rettgeri	NDM
Providencia stuartii	NDM
Pseudomonas aeruginosa	AAC, ANT, CTX-M-1, CTX-M-2, *gyr*A Mutant [c], KPC, NDM, OXA-1, PER, SHV [d], TEM [d], VEB, VIM
Raoultella ornithinolytica	KPC, NDM, OXA-48
Raoultella planticola	KPC
Serratia marcescens	CTX-M-1, CTX-M-9, KPC, NDM, OXA-48

[a] *Citrobacter freundii complex = C. freundii, C. braakii, C. werkmanii and C. youngae.*
[b] *Enterobacter cloacae complex = E. asburiae, E. cloacae, E. hormaechei, E. kobei and E. ludwigii.*
[c] *PCR assays associated with fluoroquinolone resistance detect and differentiate wild type and mutant variants of gyraseA at amino acid position 87 for E. coli and position 83 for P. aeruginosa.*
[d] *PCR assays for SHV and TEM detect several sequence variants for the two genes, respectively, at amino acid positions 156 and 104 associated with wild type penicillin resistance and mutations associated with ESBL phenotypes.*
[e] *The panel includes an assay for the detection of the mobilized colistin genetic determinant MCR-1 in E. coli.*

The Acuitas AMR Gene Panel includes assays for the detection and reporting of genetic resistance markers associated with resistance to select drugs in the following antibiotic groups: aminoglycosides, carbapenems, cephalosporins, fluoroquinolones, penicillins, sulfonamides, trimethoprim, and vancomycin, to aid in the identification of potentially antimicrobial-resistant organisms. The panel includes an assay for the detection of the mobilized colistin genetic determinant MCR-1, a marker of public health importance associated with reduced inhibitory activity of polymyxins.

[*continued on page 3*]

[*continued from page 2*]

The results of the Acuitas AMR Gene Panel for detection and identification of genetic determinants associated with antimicrobial resistance are used along with the Acuitas AMR Gene Panel Electronic User Guide (EUG). In certain cases, this information may be used as an aid to clinicians in the management of patients with known or suspected antibiotic non-susceptible or resistant bacterial infections. The EUG contains information on the appropriateness of reporting resistance markers detected by the Acuitas AMR Gene Panel for claimed organisms based on the strength of the collective, totality of scientific evidence delineating the level of association between molecular marker detection with phenotypic, clinical resistance. Test results are not conclusive or prescriptive for labeled use of any specific antimicrobial drug product, and therefore, this test cannot be used in place of or to postpone or delay phenotypic antimicrobial susceptibility testing.

A “Detected” or “Not Detected” result does not rule out the presence of other antimicrobial resistance markers not detected by the Acuitas AMR Gene Panel. A "Not Detected" result for a genetic marker of antimicrobial resistance does not indicate susceptibility to associated antimicrobial drugs or drug classes, as multiple mechanisms of resistance may exist.

510(K) SUMMARY

OpGen, Inc.

Acuitas® AMR Gene Panel

I. INTRODUCTION

This summary of 510(k) safety and effectiveness information is being submitted in accordance with the requirements of 21 CFR 807.92. According to the requirements of 21 CFR 807.92, the following information provides sufficient detail to understand the basis for a determination of substantial equivalence. The assigned 510(k) number is K191288.

A. SUBMITTER

OpGen, Inc.
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Rockville, MD 20850

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Phone: 301-869-9683
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Date Prepared: September 29, 2021

B. NAME OF DEVICE

Acuitas® AMR Gene Panel

C. COMMON OR USUAL NAME

Acuitas AMR Gene Panel

D. REGULATORY INFORMATION

1. Regulation Section:

21 CFR 866.1640 (Antimicrobial susceptibility test powder)

2. Classification:

Class II

3. Product Code:

PMY - System, Nucleic Acid Amplification Test, DNA, Carbapenem Non-Susceptible Gram Negative Organism, Colony

OOI - Real-time nucleic acid amplification system

E. PREDICATE DEVICE

The Acuitas AMR Gene Panel is substantially equivalent to the Cepheid Xpert® Carba-R [510(k) # K152614].

F. DEVICE DESCRIPTION

The Acuitas® AMR Gene Panel is a qualitative nucleic acid-based *in vitro* diagnostic test capable of simultaneous detection and identification of select genetic determinants of antimicrobial resistance (AMR) in isolated bacterial colonies grown on blood agar or MacConkey agar. The test detects twenty-eight (28) genetic determinants of resistance to the following nine (9) antibiotic classes: aminoglycosides, carbapenems, cephalosporins, fluoroquinolones, penicillins, polymyxins, sulfonamides, trimethoprim and vancomycin. The assay is performed on pure colonies of Enterobacterales and *Pseudomonas aeruginosa* grown on blood agar or MacConkey agar along with *Enterococcus faecalis* grown on blood agar.

The Acuitas AMR Gene Panel kit contains all of the necessary reagents for PCR and detection in order to amplify and detect DNA from pure colonies of Enterobacterales (*Citrobacter freundii* complex (*Citrobacter braakii*, *Citrobacter freundii*, *Citrobacter werkmanii*, *Citrobacter youngae*), *Citrobacter koseri*, *Enterobacter cloacae* complex (*Enterobacter asburiae*, *Enterobacter cloacae, Enterobacter hormaechei, Enterobacter kobei, Enterobacter ludwigii*), *Escherichia coli, Klebsiella pneumoniae, Klebsiella quasipneumoniae, Klebsiella aerogenes, Klebsiella michiganensis, Klebsiella oxytoca, Klebsiella variicola, Morganella morganii, Proteus mirabilis, Providencia rettgeri, Providencia stuartii, Raoultella ornithinolytica, Raoultella planticola, Serratia marcescens*) and *Pseudomonas aeruginosa* grown on blood agar or MacConkey agar, as well as *Enterococcus faecalis* grown on blood agar from clinical specimens. The test kit includes PCR plates (96-well) with dried primers and probes for analysis of four (4) isolates (24 wells per isolate).

The Acuitas AMR Gene Panel assay employs automated deoxyribonucleic acid (DNA) extraction on the QIAGEN® EZ1® Advanced XL System and multiplex real-time PCR on an OpGen Qualified Applied Biosystems™ QuantStudio™ 5 Real-Time PCR System (“OpGen Qualified QuantStudio 5”)

for use with the Acuitas AMR Gene Panel. The QIAGEN EZ1 DSP Virus Kit has been selected for use as the sample preparation method for the Acuitas AMR Gene Panel test.

After colony isolation, the user prepares a 0.5 McFarland suspension for each bacterial isolate and performs DNA extraction. DNA is extracted on the QIAGEN EZ1 Advanced XL System according to manufacturer instructions incorporating the Assay Control within the extraction process. A sample of extracted DNA eluate is transferred to a Reagent Reservoir trough to which Acuitas AMR Gene Panel Master Mix is added. Extracted DNA/Master Mix is transferred to each of 24 wells on the Acuitas AMR Gene Panel PCR plate per test sample. The contents of each well are mixed, and the plate is sealed and transferred to the OpGen Qualified QuantStudio 5 for use with the Acuitas AMR Gene Panel for real-time multiplex reaction and detection using the Acuitas AMR Gene Panel PCR Template File.

Data are exported from the OpGen Qualified QuantStudio 5 and imported into the Acuitas AMR Gene Analysis Software, a spreadsheet application that analyzes the data and generates a report for viewing and printing. Each test report indicates detection of applicable antimicrobial resistance gene variants as “Detected”, “Not Detected” or “NA/NR”.

The Applied Biosystems QuantStudio 5 Real-Time PCR System is not intended for clinical diagnostic purposes. The OpGen Qualified QuantStudio 5 for use with the Acuitas AMR Gene Panel is a component of the Acuitas AMR Gene Panel assay and is cleared for *in vitro* diagnostic use only with the Acuitas AMR Gene Panel and not for any other application. The OpGen Qualified QuantStudio 5 for use with the Acuitas AMR Gene Panel may only be used with the Acuitas AMR Gene Panel after the instrument has been qualified for use by OpGen, Inc.

G. INTENDED USE / INDICATIONS FOR USE

The Acuitas® AMR Gene Panel, performed on the QIAGEN® EZ1® Advanced XL System and the OpGen Qualified QuantStudio™ 5 Real-Time PCR System, is a qualitative nucleic acid-based multiplex *in vitro* diagnostic test for detection and differentiation of antibiotic resistance markers to one or more antimicrobial agents. The test utilizes real-time polymerase chain reaction (PCR) and is performed on isolated colonies of *Pseudomonas aeruginosa*, *Enterococcus faecalis, or members of Enterobacterales grown in pure culture on blood agar or MacConkey agar*.

Organism identification results must be available prior to reporting results for the Acuitas AMR Gene Panel. Antimicrobial resistance gene results are reported by the Acuitas AMR Gene Panel for the combinations of bacterial pathogens and associated genetic resistance markers indicated in *Table 1* below.

Table 1 - Antimicrobial Resistance Gene Markers (Genetic Determinants) Associated with Bacterial Species

Organism	Reported AMR Gene Marker
Citrobacter freundii complex [a]	CTX-M-1, CTX-M-9, KPC, NDM, OXA-48
Citrobacter koseri	KPC, OXA-48
Enterobacter cloacae complex [b]	CTX-M-1, CTX-M-9, KPC, TEM [d]
Enterococcus faecalis	vanA
Escherichia coli	AAC, ANT, CMY, CTX-M-1, CTX-M-2, CTX-M-9, DFR, *gyr*A Mutant [c], KPC, MCR-1 [e], OXA-1, OXA-9, SHV [d], Sul1, Sul2, TEM [d]
Klebsiella aerogenes	CTX-M-1, CTX-M-9, KPC, NDM, OXA-48
Klebsiella michiganensis	CTX-M-1, CTX-M-9, KPC, NDM, OXA-48

Organism	Reported AMR Gene Marker
Klebsiella oxytoca	CTX-M-1, CTX-M-9, KPC, NDM, OXA-48
Klebsiella pneumoniae	AAC, AAD, APH, CMY, CTX-M-1, CTX-M- 9, DFR, DHA, IMP, KPC, NDM, OXA-1, OXA-9, OXA-48, RMT, Sul1, Sul2, TEM [d]
Klebsiella quasipneumoniae	CTX-M-1, CTX-M-9, KPC, NDM, OXA-48
Klebsiella variicola	CTX-M-1, CTX-M-9, KPC, NDM, OXA-48
Morganella morganii	CTX-M-1, KPC, NDM, OXA-48
Proteus mirabilis	AAC, ANT, APH, armA, CMY, CTX-M-1, CTX-M-2, CTX-M-9, DFR, KPC, NDM, OXA-1, OXA-9, OXA-48, Sul2, TEM [d], VEB, VIM
Providencia rettgeri	NDM
Providencia stuartii	NDM
Pseudomonas aeruginosa	AAC, ANT, CTX-M-1, CTX-M-2, *gyr*A Mutant [c], KPC, NDM, OXA-1, PER, SHV [d], TEM [d], VEB, VIM
Raoultella ornithinolytica	KPC, NDM, OXA-48
Raoultella planticola	KPC
Serratia marcescens	CTX-M-1, CTX-M-9, KPC, NDM, OXA-48

[a] Citrobacter freundii complex = C. freundii, C. braakii, C. werkmanii and C. youngae.
[b] Enterobacter cloacae complex = E. asburiae, E. cloacae, E. hormaechei, E. kobei and E. ludwigii.
[c] PCR assays associated with fluoroquinolone resistance detect and differentiate wild type and mutant variants of gyraseA at amino acid position 87 for E. coli and position 83 for P. aeruginosa.
[d] PCR assays for SHV and TEM detect several sequence variants for the two genes, respectively, at amino acid positions 156 and 104 associated with wild type penicillin resistance and mutations associated with ESBL phenotypes.
[e] The panel includes an assay for the detection of the mobilized colistin genetic determinant MCR-1 in E. coli.

The Acuitas AMR Gene Panel includes assays for the detection and reporting of genetic resistance markers associated with resistance to select drugs in the following antibiotic groups: aminoglycosides, carbapenems, cephalosporins, fluoroquinolones, penicillins, sulfonamides, trimethoprim, and vancomycin, to aid in the identification of potentially antimicrobial-resistant organisms. The panel includes an assay for the detection of the mobilized colistin genetic determinant MCR-1, a marker of public health importance associated with reduced inhibitory activity of polymyxins.

The results of the Acuitas AMR Gene Panel for detection and identification of genetic determinants associated with antimicrobial resistance are used along with the Acuitas AMR Gene Panel Electronic User Guide (EUG). In certain cases, this information may be used as an aid to clinicians in the management of patients with known or suspected antibiotic non-susceptible or resistant bacterial infections. The EUG contains information on the appropriateness of reporting resistance markers detected by the Acuitas AMR Gene Panel for claimed organisms based on the strength of the collective, totality of scientific evidence delineating the level of association between molecular marker detection with phenotypic, clinical resistance. Test results are not conclusive or prescriptive for labeled use of any specific antimicrobial drug product, and therefore, this test cannot be used in place of or to postpone or delay phenotypic antimicrobial susceptibility testing.

A “Detected” or “Not Detected” result does not rule out the presence of other antimicrobial resistance markers not detected by the Acuitas AMR Gene Panel. A "Not Detected" result for a genetic marker of antimicrobial resistance does not indicate susceptibility to associated antimicrobial drugs or drug classes, as multiple mechanisms of resistance may exist.

H. PREDICATE DEVICE

Acuitas AMR Gene Panel assay is substantially equivalent to Cepheid Xpert® Carba-R, [510(k) K152614]. The Acuitas AMR Gene Panel assay and the Xpert Carba-R Assay both detect target gene sequences from antibiotic-resistant bacteria and use real-time PCR amplification and fluorogenic target-specific hybridization detection.

The performance characteristics of the Acuitas AMR Gene Panel with bacterial isolates were determined in a multi-site investigational clinical study by comparing the Acuitas AMR Gene Panel to the results of Whole Genome Sequencing (WGS) and Antimicrobial Susceptibility Testing (AST). *Table 2* compares the Acuitas AMR Gene Panel to the Cepheid Xpert® Carba-R and outlines the similarities and differences between the two systems.

Table 2 - Comparison of Similarities and Differences of the Acuitas AMR Gene Panel with the Predicate Device

Item	**Proposed Device**	**Predicate Device**
	Acuitas® AMR Gene Panel	**Xpert® Carba-R Assay (K152614)**
Sample Types	Bacterial Isolates	Bacterial Isolates
Organisms Indicated	Enterobacterales*, Pseudomonas aeruginosa, and Enterococcus faecalis*	*Enterobacteriaceae, Acinetobacter baumannii, Pseudomonas aeruginosa*
Assay Targets	AAC, AAD, ANT, APH, armA, CMY, CTX-M-1, CTX-M-2, CTX-M-9, DFR, DHA, *E. coli gyr*A mutant, IMP, KPC, MCR-1, NDM, OXA-1, OXA-9, OXA-48, *P. aeruginosa gyr*A mutant, PER, RMT, SHV, Sul1, Sul2, TEM, vanA, VEB and VIM	blaKPC, blaNDM, blaVIM, blaOXA-48, and blaIMP
Similarities		
Result Types	Qualitative	Same
Analyte	DNA	Same
Technological Principles	Automated Nucleic acid amplification (DNA); real-time PCR	Fully automated nucleic acid amplification (DNA); real-time PCR
Interpretation of Results	Diagnostic software on a Personal Computer (PC)	Same
Differences		
Organism Detected	Enterobacterales*, Pseudomonas aeruginosa and Enterococcus faecalis*	*Enterobacteriaceae, Acinetobacter baumannii, Pseudomonas aeruginosa*
Target Antibiotic Classes	Aminoglycosides, Carbapenems, Cephalosporins, Fluoroquinolones, Penicillins, Polymyxins, Sulfonamides, Trimethoprim, and Vancomycin	Carbapenems

Item	Proposed Device	Predicate Device
	Acuitas® AMR Gene Panel	**Xpert® Carba-R Assay (K152614)**
Gene Sequence	AAC, AAD, ANT, APH, armA, CMY, CTX-M-1, CTX-M-2, CTX-M-9, DFR, DHA, *E. coli gyr*A mutant, IMP, KPC, MCR-1, NDM, OXA-1, OXA-9, OXA-48, *P. aeruginosa gyr*A mutant, PER, RMT, SHV, Sul1, Sul2, TEM, vanA, VEB and VIM	blaKPC, blaNDM, blaVIM, blaOXA-48, and blaIMP
Extraction Method	QIAGEN® EZ1® Advanced XL DNA Extraction	Integrated with GeneXpert® System
Instrument	OpGen Qualified QuantStudio™ 5 Real-Time PCR System[1]	Cepheid GeneXpert® System
Controls	One Assay Control, External Controls Available	Internal Sample Processing Control (SPC) and Probe Check Control (PCC); External Controls available
Software	Spreadsheet application which takes output from the OpGen Qualified QuantStudio 5 and generates report on the gene sequence	Automated test report using diagnostic software
Time to obtain results from start of test	Approximately 2.5 hours	Approximately 50 minutes to results

[1] The Applied Biosystems QuantStudio 5 Real-Time PCR System is not intended for clinical diagnostic purposes. The OpGen Qualified QuantStudio 5 for use with the Acuitas AMR Gene Panel is a component of the Acuitas AMR Gene Panel assay and is cleared for *in vitro* diagnostic use only with the Acuitas AMR Gene Panel and not for any other application. The OpGen Qualified QuantStudio 5 for use with the Acuitas AMR Gene Panel may only be used with the Acuitas AMR Gene Panel after the instrument has been qualified for use by OpGen, Inc.

II. PERFORMANCE DATA

A. SELECTED NON-CLINICAL STUDIES

1. Reproducibility

Reproducibility of the Acuitas AMR Gene Panel was evaluated using a panel of 300 uniquely labeled samples composed of ten (10) unique isolates. The panel was provided to three (3) testing sites, one (1) of which was OpGen. The panel was rotated across two (2) operators, two (2) OpGen Qualified QuantStudio 5 instruments and one (1) EZ1 Instrument per site over 20 days. Three (3) unique lots of all materials were used and rotated at each testing site.

Overall results for each sample tested in the reproducibility study are summarized in *Table 3 - Acuitas AMR Gene Panel Reproducibility of Study Panel – by Isolate*. Total agreement per sample (All Sites) ranged from 96% to 100%.

Table 3 - Acuitas AMR Gene Panel Reproducibility of Study Panel – by Isolate [a]

Isolate	Site 1			Site 2			Site 3			All Sites
	Op1	Op2	Site 1	Op1	Op2	Site 2	Op1	Op2	Site 3	
L00000068-001 *(E. coli)*	14/15 93%	15/15 100%	29/30 97%	14/15 93%	14/15 93%	28/30 93%	15/15 100%	14/15 93%	29/30 97%	86/90 96%
L00015886-001 *(E. coli)*	13/14[b] 93%	15/15 100%	28/29 97%	15/15 100%	15/15 100%	30/30 100%	15/15 100%	15/15 100%	30/30 100%	88/89 99%
L00009154-001 *(E. coli)*	15/15 100%	15/15 100%	30/30 100%	15/15 100%	14/15 93%	29/30 97%	15/15 100%	15/15 100%	30/30 100%	89/90 99%
L00009721-001 *(K. pneumoniae)*	15/15 100%	15/15 100%	30/30 100%	15/15 100%	14/15 93%	29/30 97%	14/15 93%	15/15 100%	29/30 97%	88/90 98%
L00007800-001 *(K. pneumoniae)*	15/15 100%	15/15 100%	30/30 100%	15/15 100%	14/15 93%	29/30 97%	15/15 100%	14/15 93%	29/30 97%	88/90 98%
L00008624-001 *(P. aeruginosa)*	15/15 100%	14/15 93%	29/30 97%	15/15 100%	15/15 100%	30/30 100%	14/15 93%	14/15[c] 93%	28/30 93%	87/90 97%
L00013504-001 *(P. mirabilis)*	15/15 100%	15/15 100%	30/30 100%	15/15 100%	14/15 93%	29/30 97%	15/15 100%	14/15 93%	29/30 97%	88/90 98%
L00013200-001[d] *(P. mirabilis)*	15/15 100%	15/15 100%	30/30 100%	14/15 93%	14/14[e] 100%	28/29 97%	15/15 100%	15/15 100%	30/30 100%	88/89 99%
L00022926-001 *(E. faecalis)*	15/15 100%	15/15 100%	30/30 100%	15/15 100%	15/15 100%	30/30 100%	15/15 100%	15/15 100%	30/30 100%	90/90 100%
L00006246-001[f] *(S. aureus)*	15/15 100%	15/15 100%	30/30 100%	15/15 100%	15/15 100%	30/30 100%	15/15 100%	14/15[f] 93%	29/30 97%	89/90 99%

a. Table includes reported AMR gene results per organism as described in Table 1 along with species ID results for *E. coli* and *P. aeruginosa* as used in conjunction with mutant gyrase results for these two organisms.

b. One replicate of L00015886-001 had invalid results due to an Assay Control Failure. The replicate was repeated using the same lot, instrument, and operator as the original testing event. The repeat data agreed 100% with expected results. Neither initial nor repeat results are reported for this replicate.

c. Amplification present for *P. aeruginosa gyraseA* assay called negative due to negative *P. aeruginosa* ID result in one sample.

d. CTX-M-14 (AF252622) was reported by WGS for L00013200-001-300187 on lane 300187, which are the correct WGS results for L00013200-001.

e. One replicate of L00013200-001 had invalid results due to an Assay Control Failure. The replicate was repeated using the same lot, instrument, and operator as the original testing event. The repeat data had 100% agreement with the expected results. Neither initial nor repeat results are reported for this replicate.

f. The Acuitas AMR Gene Panel is not intended for *S. aureus*, which served as a negative control in this study. *S. aureus* was evaluated for all AMR genes in Table 1 except for gyrase gene targets. One replicate sample of L00006246-001 was false positive for the AAC assay.

Overall results for each assay target in the reproducibility test panel are summarized in *Table 4 - Acuitas AMR Gene Panel Reproducibility of Study Panel - by Gene Target.* Total agreement ranged from 97.8% to 100% for detected results and 99.4% to 100% for not detected results across the individual assay targets.

Table 4 - Acuitas AMR Gene Panel Reproducibility of Study Panel - by Gene Target [d]

Acuitas AMR Gene Target	Expected Results	Site 1			Site 2			Site 3			Total % Agreement by Target
		Op 1 [a]	Op 2	Site	Op 1	Op 2 [b]	Site	Op 1	Op 2	Site	
AAC	Detected	60/60 100%	60/60 100%	120/120 100%	60/60 100%	59/59 100%	119/119 100%	60/60 100%	60/60 100%	120/120 100%	359/359 100%
	Not Detected	74/74 100%	75/75 100%	149/149 100%	75/75 100%	75/75 100%	150/150 100%	75/75 100%	74/75 [d] 98.7%	149/150 99.3%	448/449 99.80%
AAD	Detected	15/15 100%	15/15 100%	30/30 100%	15/15 100%	15/15 100%	30/30 100%	15/15 100%	15/15 100%	30/30 100%	90/90 100%
	Not Detected	30/30 100%	30/30 100%	60/60 100%	30/30 100%	30/30 100%	60/60 100%	30/30 100%	30/30 100%	60/60 100%	180/180 100%
ANT	Detected	30/30 100%	30/30 100%	60/60 100%	29/30 96.7%	30/30 100%	59/60 98.30%	30/30 100%	28/30 93.3%	58/60 96.7%	177/180 98.30%
	Not Detected	74/74 100%	75/75 100%	149/149 100%	75/75 100%	73/74 98.6%	148/149 99.3%	74/75 98.7%	75/75 100%	149/150 99.3%	446/448 99.60%
APH	Detected	15/15 100%	15/15 100%	30/30 100%	15/15 100%	14/14 100%	29/29 100%	15/15 100%	15/15 100%	30/30 100%	89/89 100%
	Not Detected	60/60 100%	60/60 100%	120/120 100%	60/60 100%	60/60 100%	120/120 100%	60/60 100%	60/60 100%	120/120 100%	360/360 100%
armA	Detected [e]	-	-	-	-	-	-	-	-	-	-
	Not Detected	45/45 100%	45/45 100%	90/90 100%	45/45 100%	44/44 100%	89/89 100%	45/45 100%	45/45 100%	90/90 100%	269/269 100%
CMY	Detected	30/30 100%	30/30 100%	60/60 100%	30/30 100%	30/30 100%	60/60 100%	30/30 100%	30/30 100%	60/60 100%	180/180 100%
	Not Detected	88/89 98.9%	90/90 100%	178/179 99.4%	90/90 100%	89/89 100%	179/179 100%	90/90 100%	90/90 100%	180/180 100%	537/538 99.80%
CTX-M-1	Detected	30/30 100%	30/30 100%	60/60 100%	30/30 100%	30/30 100%	60/60 100%	30/30 100%	30/30 100%	60/60 100%	180/180 100%
	Not Detected	104/104 100%	105/105 100%	209/209 100%	105/105 100%	104/104 100%	209/209 100%	105/105 100%	105/105 100%	210/210 100%	628/628 100%
CTX-M-2	Detected [e]	-	-	-	-	-	-	-	-	-	-
	Not Detected	104/104 100%	105/105 100%	209/209 100%	105/105 100%	104/104 100%	209/209 100%	105/105 100%	105/105 100%	210/210 100%	628/628 100%
CTX-M-9	Detected	15/15 100%	15/15 100%	30/30 100%	15/15 100%	14/14 100%	29/29 100%	15/15 100%	15/15 100%	30/30 100%	89/89 100%
	Not Detected	104/104 100%	105/105 100%	209/209 100%	105/105 100%	104/105 99%	209/210 99.5%	105/105 100%	105/105 100%	210/210 100%	628/629 99.80%
DFR	Detected	45/45 100%	45/45 100%	90/90 100%	45/45 100%	44/44 100%	89/89 100%	45/45 100%	45/45 100%	90/90 100%	269/269 100%
	Not Detected	74/74 100%	75/75 100%	149/149 100%	75/75 100%	75/75 100%	150/150 100%	75/75 100%	75/75 100%	150/150 100%	449/449 100%
DHA	Detected [e]	-	-	-	-	-	-	-	-	-	-

Acuitas AMR Gene Target	Expected Results	Site 1			Site 2			Site 3			Total % Agreement by Target
		Op 1 [a]	Op 2	Site	Op 1	Op 2 [b]	Site	Op 1	Op 2	Site	
	Not Detected	45/45 100%	45/45 100%	90/90 100%	45/45 100%	45/45 100%	90/90 100%	45/45 100%	45/45 100%	90/90 100%	270/270 100%
*E. coli gyr*A Mutant	Detected	14/15 93.3%	15/15 100%	29/30 96.7%	15/15 100%	14/15 93.3%	29/30 96.70%	15/15 100%	15/15 100%	30/30 100%	88/90 97.80%
	Not Detected	29/29 100%	30/30 100%	59/59 100%	30/30 100%	30/30 100%	60/60 100%	30/30 100%	30/30 100%	60/60 100%	179/179 100%
E. coli ID [f]	Detected	44/44 100%	45/45 100%	89/89 100%	45/45 100%	44/45 97.8%	89/90 98.9%	45/45 100%	45/45 100%	90/90 100%	268/269 99.6%
	Not Detected [e]	-	-	-	-	-	-	-	-	-	-
IMP	Detected	15/15 100%	15/15 100%	30/30 100%	15/15 100%	15/15 100%	30/30 100%	15/15 100%	15/15 100%	30/30 100%	90/90 100%
	Not Detected	30/30 100%	30/30 100%	60/60 100%	30/30 100%	30/30 100%	60/60 100%	29/30 96.7%	30/30 100%	59/60 98.3%	179/180 99.40%
KPC	Detected	15/15 100%	15/15 100%	30/30 100%	15/15 100%	15/15 100%	30/30 100%	15/15 100%	15/15 100%	30/30 100%	90/90 100%
	Not Detected	119/119 100%	120/120 100%	239/239 100%	120/120 100%	119/119 100%	239/239 100%	120/120 100%	120/120 100%	240/240 100%	718/718 100%
MCR-1	Detected [e]	-	-	-	-	-	-	-	-	-	-
	Not Detected	59/59 100%	60/60 100%	119/119 100%	60/60 100%	60/60 100%	120/120 100%	60/60 100%	60/60 100%	120/120 100%	359/359 100%
NDM	Detected	15/15 100%	15/15 100%	30/30 100%	15/15 100%	15/15 100%	30/30 100%	15/15 100%	15/15 100%	30/30 100%	90/90 100%
	Not Detected	75/75 100%	75/75 100%	150/150 100%	75/75 100%	74/74 100%	149/149 100%	75/75 100%	75/75 100%	150/150 100%	449/449 100%
OXA-1	Detected	30/30 100%	30/30 100%	60/60 100%	30/30 100%	29/29 100%	59/59 100%	30/30 100%	30/30 100%	60/60 100%	179/179 100%
	Not Detected	104/104 100%	105/105 100%	209/209 100%	105/105 100%	105/105 100%	210/210 100%	105/105 100%	105/105 100%	210/210 100%	629/629 100%
OXA-9	Detected [e]	-	-	-	-	-	-	-	-	-	-
	Not Detected	119/119 100%	120/120 100%	239/239 100%	120/120 100%	119/119 100%	239/239 100%	120/120 100%	120/120 100%	240/240 100%	718/718 100%
OXA-48	Detected	15/15 100%	15/15 100%	30/30 100%	15/15 100%	15/15 100%	30/30 100%	15/15 100%	15/15 100%	30/30 100%	90/90 100%
	Not Detected	60/60 100%	60/60 100%	120/120 100%	59/60 98.3%	59/59 100%	118/119 99.2%	60/60 100%	60/60 100%	120/120 100%	358/359 99.70%
*P. aeruginosa gyr*A Mutant	Detected	15/15 100%	14/15 93.3%	29/30 96.7%	15/15 100%	15/15 100%	30/30 100%	15/15 100%	14/15 [c] 93.3%	29/30 96.7%	88/90 97.80%
	Not Detected [e]	-	-	-	-	-	-	-	-	-	-

Acuitas AMR Gene Target	Expected Results	Site 1			Site 2			Site 3			Total % Agreement by Target
		Op 1 [a]	Op 2	Site	Op 1	Op 2 [b]	Site	Op 1	Op 2	Site	
P. aeruginosa ID [f]	Detected	15/15 100%	15/15 100%	30/30 100%	15/15 100%	15/15 100%	30/30 100%	15/15 100%	14/15 93.3%	29/30 96.7%	89/90 98.9%
	Not Detected [e]	-	-	-	-	-	-	-	-	-	-
PER	Detected	15/15 100%	15/15 100%	30/30 100%	15/15 100%	15/15 100%	30/30 100%	15/15 100%	15/15 100%	30/30 100%	90/90 100%
	Not Detected	15/15 100%	15/15 100%	30/30 100%	15/15 100%	15/15 100%	30/30 100%	15/15 100%	15/15 100%	30/30 100%	90/90 100%
RMT	Detected [e]	-	-	-	-	-	-	-	-	-	-
	Not Detected	45/45 100%	45/45 100%	90/90 100%	45/45 100%	45/45 100%	90/90 100%	45/45 100%	45/45 100%	90/90 100%	270/270 100%
SHV	Detected [e]	-	-	-	-	-	-	-	-	-	-
	Not Detected	74/74 100%	75/75 100%	149/149 100%	75/75 100%	75/75 100%	150/150 100%	75/75 100%	75/75 100%	150/150 100%	449/449 100%
Sul1	Detected	60/60 100%	60/60 100%	120/120 100%	60/60 100%	60 /60 100%	120/120 100%	60/60 100%	60/60 100%	120/120 100%	360/360 100%
	Not Detected	29/29 100%	30/30 100%	59/59 100%	30/30 100%	30/30 100%	60/60 100%	30/30 100%	30/30 100%	60/60 100%	179/179 100%
Sul2	Detected	104/104 100%	105/105 100%	209/209 100%	105/105 100%	103/104 99%	208/209 99.5%	105/105 100%	105/105 100%	210/210 100%	627/628 99.80%
	Not Detected	15/15 100%	15/15 100%	30/30 100%	15/15 100%	15/15 100%	30/30 100%	15/15 100%	15/15 100%	30/30 100%	90/90 100%
TEM	Detected	104/104 100%	105/105 100%	209/209 100%	105/105 100%	103/104 99%	208/209 99.5%	105/105 100%	105/105 100%	210/210 100%	627/628 99.80%
	Not Detected	30/30 100%	30/30 100%	60/60 100%	30/30 100%	30/30 100%	60/60 100%	30/30 100%	30/30 100%	60/60 100%	180/180 100%
vanA	Detected	15/15 100%	15/15 100%	30/30 100%	15/15 100%	15/15 100%	30/30 100%	15/15 100%	15/15 100%	30/30 100%	90/90 100%
	Not Detected	15/15 100%	15/15 100%	30/30 100%	15/15 100%	15/15 100%	30/30 100%	15/15 100%	15/15 100%	30/30 100%	90/90 100%
VEB	Detected	15/15 100%	15/15 100%	30/30 100%	15/15 100%	15/15 100%	30/30 100%	15/15 100%	15/15 100%	30/30 100%	90/90 100%
	Not Detected	45/45 100%	45/45 100%	90/90 100%	45/45 100%	44/44 100%	89/89 100%	45/45 100%	45/45 100%	90/90 100%	269/269 100%
VIM	Detected	15/15 100%	15/15 100%	30/30 100%	15/15 100%	15/15 100%	30/30 100%	15/15 100%	15/15 100%	30/30 100%	90/90 100%
	Not Detected	45/45 100%	45/45 100%	90/90 100%	45/45 100%	44/44 100%	89/89 100%	45/45 100%	45/45 100%	90/90 100%	269/269 100%

[a] One replicate of L00015886-001 had invalid results due to an Assay Control Failure. The replicate was repeated using the same lot, instrument, and operator as the original testing event. The repeat data had 100% agreement with the expected results. Neither initial nor repeat results are reported for this replicate.

[b] One replicate of L00013200-001 had invalid results due to an Assay Control Failure. The replicate was repeated using the same lot, instrument, and operator as the original testing event. The repeat data had 100% agreement with the expected results. Neither initial nor repeat results are reported for this replicate.

[c] Amplification present for *P. aeruginosa gyrase*A assay called negative due to negative *P. aeruginosa* ID result in one sample.

[d] The Acuitas AMR Gene Panel is not intended for *S. aureus*, which served as a negative control in this study. *S. aureus* was evaluated for all AMR genes in Table 3 except for gyrase gene targets. One replicate sample of L00006246-001 was false positive for the AAC assay.
[e] No data available.
[f] Organism ID only utilized in the context of *gyr*A mutant detection.

2. Analytical Reactivity (Inclusivity)

The analytical sensitivity of the Acuitas AMR Gene Panel was evaluated by testing a panel of two hundred and ninety-eight (298) isolates covering all antimicrobial resistance genes detected by the Acuitas AMR Gene Panel test. Each isolate in the test panel was tested once, except for isolates harboring rare resistance genes, which were replicated to achieve at least 18 positive data points per gene target assessed. The Acuitas AMR Gene Panel results were compared with species identification by well-established automated species identification methods and AMR gene detection by Whole Genome Sequencing (WGS).

Table 5 - Analytical Reactivity (Inclusivity) Results summarizes AMR genes detected or not detected by Acuitas AMR Gene Panel. *Table 5* also indicates AMR gene variants predicted to be detected by *in silico* analysis but not tested in this study.

Table 5 - Analytical Reactivity (Inclusivity) Results [a, e]

AMR Gene	Acuitas AMR Gene Panel				Other AMR Gene Variants Predicted to be Detected by Acuitas AMR Gene Panel Based on *In Silico* Analysis[d]
	Number of Samples Positive for Gene by WGS	Number of Unique Isolates Positive for Gene by WGS	AMR Gene(s) Detected	AMR Gene(s) Not Detected	
AAC	198	162	aac(3)-IIa, aac(3)-IId, aac(3)-IVa, aac(6')-Ib, aac(6')Ib-cr, aacA4-8, aacA4		Detectable: aac(3)-IIc, aac(3)-IIe, aac(3)-Ib-aac(6')-Ib, ant(3")-Ih-aac(6')-IId
AAD	70	62	aadA1, aadA2		Detectable: aadA13, aadA3, aadA8, aadA8b, aadA7[b], aadA17 Likely Detectable: aadA12, aadA21, aadA22, aadA23
ANT	63	40	aadB		-
APH	15	14	aph(4)-Ia	aph(4)-Ia_V01499	-
armA	1	1	armA		-
CMY	26	25	blaCMY-16, blaCMY-2, blaCMY-4, blaCMY-42, blaCMY-6, blaCMY-60	blaCMY-16_FJ855437	Detectable: blaBIL-1, blaCMY-0, blaCMY-102, blaCMY-108, blaCMY-110, blaCMY-111, blaCMY-112, blaCMY-113, blaCMY-114, blaCMY-115, blaCMY-118, blaCMY-12, blaCMY-14, blaCMY-15, blaCMY-17, blaCMY-18, blaCMY-20, blaCMY-21, blaCMY-22, blaCMY-23, blaCMY-24, blaCMY-25, blaCMY-27, blaCMY-28, blaCMY-29, blaCMY-3, blaCMY-30, blaCMY-31, blaCMY-32, blaCMY-33, blaCMY-34, blaCMY-35, blaCMY-36, blaCMY-38, blaCMY-39, blaCMY-41, blaCMY-43, blaCMY-44, blaCMY-45, blaCMY-47, blaCMY-48, blaCMY-5, blaCMY-50, blaCMY-51, blaCMY-54, blaCMY-55, blaCMY-56, blaCMY-57, blaCMY-58, blaCMY-59, blaCMY-61, blaCMY-62, blaCMY-63, blaCMY-65, blaCMY-66, blaCMY-67, blaCMY-68, blaCMY-69, blaCMY-7, blaCMY-71, blaCMY-72, blaCMY-75, blaCMY-76, blaCMY-77, blaCMY-78, blaCMY-80, blaCMY-81, blaCMY-84, blaCMY-87, blaCMY-90, blaCMY-94, blaCMY-95,

AMR Gene	Acuitas AMR Gene Panel				Other AMR Gene Variants Predicted to be Detected by Acuitas AMR Gene Panel Based on *In Silico* Analysis[d]
	Number of Samples Positive for Gene by WGS	Number of Unique Isolates Positive for Gene by WGS	AMR Gene(s) Detected	AMR Gene(s) Not Detected	
					blaCMY-99, blaLAT-1, blaCMY-103, blaCMY-117, blaCMY-79 Likely Detectable: blaCMY-13, blaCMY-26, blaCMY-37, blaCMY-49, blaCMY-73, blaCMY-79, blaCMY-116, blaCMY-117, Potentially Detectable: blaCMY-40, blaCMY-53
CTX-M-1	72	62	blaCTX-M-1, blaCTX-M-15, blaCTX-M-55, blaCTX-M-64	blaCTX-M-15_DQ302097	Detectable: blaCTX-M, blaCTX-M-10, blaCTX-M-101, blaCTX-M-103, blaCTX-M-107, blaCTX-M-108, blaCTX-M-109, blaCTX-M-11, blaCTX-M-114, blaCTX-M-117, blaCTX-M-12, blaCTX-M-123, blaCTX-M-132, blaCTX-M-136, blaCTX-M-139, blaCTX-M-144, blaCTX-M-22, blaCTX-M-28, blaCTX-M-29, blaCTX-M-3, blaCTX-M-30, blaCTX-M-32, blaCTX-M-33, blaCTX-M-34, blaCTX-M-36, blaCTX-M-37, blaCTX-M-38, blaCTX-M-42, blaCTX-M-52, blaCTX-M-53, blaCTX-M-54, blaCTX-M-58, blaCTX-M-60, blaCTX-M-61, blaCTX-M-62, blaCTX-M-66, blaCTX-M-68, blaCTX-M-69, blaCTX-M-71, blaCTX-M-72, blaCTX-M-79, blaCTX-M-82, blaCTX-M-88, blaCTX-M-89, blaCTX-M-96 Likely Detectable: blaCTX-M-116, blaCTX-M-142, blaCTX-M-23, blaCTX-M-80
CTX-M-2	14	12	blaCTX-M-131, blaCTX-M-2		Detectable: blaCTX-M-141, blaCTX-M-20, blaCTX-M-31, blaCTX-M-35, blaCTX-M-43, blaCTX-M-44, blaCTX-M-5, blaCTX-M-56, blaCTX-M-59, blaCTX-M-76, blaCTX-M-77, blaCTX-M-92, blaCTX-M-95, blaCTX-M-97 Likely Detectable: blaCTX-M-115, blaCTX-M-124
CTX-M-9	34	32	blaCTX-M-14, blaCTX-M-14b, blaCTX-M-27, blaCTX-M-64, blaCTX-M-65, blaCTX-M-9, blaCTX-M-90		Detectable: blaCTX-M-104, blaCTX-M-106, blaCTX-M-110, blaCTX-M-111, blaCTX-M-112, blaCTX-M-113, blaCTX-M-121, blaCTX-M-122, blaCTX-M-123, blaCTX-M-125, blaCTX-M-126, blaCTX-M-129, blaCTX-M-13, blaCTX-M-130, blaCTX-M-134, blaCTX-M-147, blaCTX-M-148, blaCTX-M-159, blaCTX-M-16, blaCTX-M-17, blaCTX-M-19, blaCTX-M-21, blaCTX-M-24, blaCTX-M-38, blaCTX-M-46, blaCTX-M-47 blaCTX-M-48, blaCTX-M-49, blaCTX-M-50, blaCTX-M-51, blaCTX-M-67, blaCTX-M-81, blaCTX-M-83, blaCTX-M-84, blaCTX-M-85, blaCTX-M-86, blaCTX-M-87, blaCTX-M-93, blaCTX-M-98, blaCTX-M-99
DFR	42	37	dfrA17, dfrA5		-
DHA	16	13	blaDHA-1		Detectable: blaDHA-10, blaDHA-13, blaDHA-14, blaDHA-15, blaDHA-17, blaDHA-18, blaDHA-19, blaDHA-2, blaDHA-20, blaDHA-21, blaDHA-22, blaDHA-3, blaDHA-5, blaDHA-6, blaDHA-7, blaDHA-9, blaMOR-2
IMP	25	15	blaIMP-1, blaIMP-13, blaIMP-18, blaIMP-26, blaIMP-34, blaIMP-4, blaIMP-6		Detectable: blaIMP-10, blaIMP-25, blaIMP-3, blaIMP-40, blaIMP-42, blaIMP-52, blaIMP-14, blaIMP-14a, blaIMP-19, blaIMP-2, blaIMP-20, blaIMP-24, blaIMP-32, blaIMP-48, blaIMP-8, blaIMP-28, blaIMP-5 Likely Detectable: blaIMP-15, blaIMP-29 Potentially Detectable: blaIMP-38
KPC	23	22	blaKPC-2, blaKPC-3	blaKPC-2_AY034847	Detectable: blaKPC-1, blaKPC-10, blaKPC-11, blaKPC-12, blaKPC-13, blaKPC-14, blaKPC-15, blaKPC-16, blaKPC-17, blaKPC-19, blaKPC-22, blaKPC-4, blaKPC-5, blaKPC-6, blaKPC-8, blaKPC-9
MCR-1	20	13	MCR-1		-
NDM	10	10	blaNDM-1, blaNDM-7		Detectable: blaNDM-12, blaNDM-2, blaNDM-3, blaNDM-4, blaNDM-5, blaNDM-6, blaNDM-8, blaNDM-9 Likely Detectable: blaNDM-10
OXA-1	78	56	blaOXA-1, blaOXA-4	blaOXA-1_J02967	Detectable: blaOXA-224, blaOXA-31, blaOXA-320 Likely Detectable: blaOXA-47

AMR Gene	Acuitas AMR Gene Panel				Other AMR Gene Variants Predicted to be Detected by Acuitas AMR Gene Panel Based on *In Silico* Analysis[d]
	Number of Samples Positive for Gene by WGS	Number of Unique Isolates Positive for Gene by WGS	AMR Gene(s) Detected	AMR Gene(s) Not Detected	
OXA-48	19	19	blaOXA-181, blaOXA-232, blaOXA-370, blaOXA-48	blaOXA-48_AY236073	Detectable: blaOXA-162, blaOXA-163, blaOXA-199, blaOXA-204, blaOXA-244, blaOXA-245, blaOXA-247
OXA-9	26	26	blaOXA-9	blaOXA-9_JF703130	-
PER	20	19	blaPER-1, blaPER-3		Detectable: blaPER-4, blaPER-5, blaPER-7, blaPER-8
RMT	11	9	rmtB		Detectable: rmtF Likely Detectable: rmtB2
SHV	4	3	blaSHV-12, blaSHV-2a		Detectable: blaSHV-1, blaSHV-100, blaSHV-101, blaSHV-102, blaSHV-103, blaSHV-104, blaSHV-106, blaSHV-107, blaSHV-108, blaSHV-109, blaSHV-11, blaSHV-119, blaSHV-120, blaSHV-121, blaSHV-122, blaSHV-128, blaSHV-129, blaSHV-13, blaSHV-132, blaSHV-133, blaSHV-135, blaSHV-137, blaSHV-14, blaSHV-140, blaSHV-141, blaSHV-142, blaSHV-143, blaSHV-144, blaSHV-145, blaSHV-147, blaSHV-148, blaSHV-149, blaSHV-15, blaSHV-150, blaSHV-151, blaSHV-152, blaSHV-153, blaSHV-154, blaSHV-155, blaSHV-156, blaSHV-157, blaSHV-158, blaSHV-159, blaSHV-16, blaSHV-160, blaSHV-161, blaSHV-162, blaSHV-163, blaSHV-164, blaSHV-165, blaSHV-167, blaSHV-168, blaSHV-172, blaSHV-173, blaSHV-178, blaSHV-179, blaSHV-18, blaSHV-183, blaSHV-2, blaSHV-24, blaSHV-25, blaSHV-26, blaSHV-28, blaSHV-29, blaSHV-30, blaSHV-31, blaSHV-33, blaSHV-34, blaSHV-35, blaSHV-36, blaSHV-38, blaSHV-40, blaSHV-41, blaSHV-42, blaSHV-44, blaSHV-46, blaSHV-48, blaSHV-49, blaSHV-5, blaSHV-50, blaSHV-51, blaSHV-52, blaSHV-55, blaSHV-56, blaSHV-57, blaSHV-59, blaSHV-60, blaSHV-61, blaSHV-62, blaSHV-63, blaSHV-64, blaSHV-65, blaSHV-66, blaSHV-67, blaSHV-69, blaSHV-7, blaSHV-70, blaSHV-71, blaSHV-72, blaSHV-73, blaSHV-74, blaSHV-75, blaSHV-76, blaSHV-77, blaSHV-78, blaSHV-79, blaSHV-8, blaSHV-80, blaSHV-81, blaSHV-82, blaSHV-83, blaSHV-85, blaSHV-86, blaSHV-89, blaSHV-92, blaSHV-94, blaSHV-95, blaSHV-96, blaSHV-97, blaSHV-98, blaSHV-99, blaOKP-A, blaSHV-105, blaSHV-110, blaSHV-27, blaSHV-45, blaSHV-93 Likely Detectable: blaSHV-119, blaSHV-137, blaSHV-144, blaSHV-167, blaSHV-168, blaSHV-38, blaSHV-51, blaSHV-70, blaSHV-71, blaSHV-72, blaSHV-73, blaSHV-80, blaSHV-81
Sul1	137	103	Sul1	Sul1_AY224185	Sul3 (AY047357)[c], Sul3 (AB281183)[c] Likely Detectable: Sul1 (AM746675) Potentially Detectable: Sul1 (AY260546)
Sul2	133	99	Sul2	Sul2_GQ421466	-
TEM	173	132	blaTEM-1, blaTEM-117, blaTEM-143, blaTEM-176, blaTEM-192, blaTEM-1A, blaTEM-1B, blaTEM-1C, blaTEM-1D, blaTEM-2, blaTEM-33		Detectable: blaTEM-10, blaTEM-101, blaTEM-102, blaTEM-104, blaTEM-105, blaTEM-108, blaTEM-11, blaTEM-110, blaTEM-112, blaTEM-114, blaTEM-115, blaTEM-116, blaTEM-118, blaTEM-12, blaTEM-120, blaTEM-122, blaTEM-126, blaTEM-127, blaTEM-128, blaTEM-129, blaTEM-132, blaTEM-135, blaTEM-136, blaTEM-137, blaTEM-141, blaTEM-144, blaTEM-145, blaTEM-147, blaTEM-148, blaTEM-150, blaTEM-151, blaTEM-152, blaTEM-154, blaTEM-155, blaTEM-156, blaTEM-157, blaTEM-158, blaTEM-159, blaTEM-160, blaTEM-163, blaTEM-164, blaTEM-166, blaTEM-168,

AMR Gene	Acuitas AMR Gene Panel				Other AMR Gene Variants Predicted to be Detected by Acuitas AMR Gene Panel Based on *In Silico* Analysis[d]
	Number of Samples Positive for Gene by WGS	Number of Unique Isolates Positive for Gene by WGS	AMR Gene(s) Detected	AMR Gene(s) Not Detected	
					blaTEM-169, blaTEM-171, blaTEM-178, blaTEM-182, blaTEM-183, blaTEM-185, blaTEM-186, blaTEM-187, blaTEM-188, blaTEM-189, blaTEM-190, blaTEM-193, blaTEM-194, blaTEM-195, blaTEM-198, blaTEM-20, blaTEM-201, blaTEM-206, blaTEM-207, blaTEM-209, blaTEM-216, blaTEM-217, blaTEM-28, blaTEM-29, blaTEM-30, blaTEM-34, blaTEM-45, blaTEM-47, blaTEM-48, blaTEM-49, blaTEM-53, blaTEM-54, blaTEM-55, blaTEM-57, blaTEM-67, blaTEM-68, blaTEM-70, blaTEM-71, blaTEM-72, blaTEM-75, blaTEM-76, blaTEM-77, blaTEM-78, blaTEM-79, blaTEM-80, blaTEM-81, blaTEM-82, blaTEM-83, blaTEM-84, blaTEM-85, blaTEM-86, blaTEM-91, blaTEM-93, blaTEM-95, blaTEM-96, blaTEM-97, blaTEM-98, blaTEM-99, blaTEM-106, blaTEM-107, blaTEM-109, blaTEM-111, blaTEM-113, blaTEM-121, blaTEM-123, blaTEM-124, blaTEM-130, blaTEM-131, blaTEM-133, blaTEM-134, blaTEM-138, blaTEM-139, blaTEM-142, blaTEM-149, blaTEM-15, blaTEM-153, blaTEM-16, blaTEM-167, blaTEM-177, blaTEM-184, blaTEM-197, blaTEM-199, blaTEM-205, blaTEM-21, blaTEM-211, blaTEM-22, blaTEM-24, blaTEM-3, blaTEM-43, blaTEM-52, blaTEM-52B, blaTEM-52C, blaTEM-6, blaTEM-60, blaTEM-63, blaTEM-8, blaTEM-87, blaTEM-88, blaTEM-89, blaTEM-92, blaTEM-94 Likely Detectable: blaTEM-17, blaTEM-125, blaTEM-146, blaTEM-90
vanA	21	4	VanA-A		-
VEB	14	13	blaVEB-1, blaVEB-5, blaVEB-6		Detectable: blaVEB-2, blaVEB-3, blaVEB-4, blaVEB-7, blaVEB-8
VIM	20	18	blaVIM-1, blaVIM-2, blaVIM-20		Detectable: blaVIM-12, blaVIM-13, blaVIM-14, blaVIM-19, blaVIM-26, blaVIM-27, blaVIM-28, blaVIM-29, blaVIM-32, blaVIM-33, blaVIM-34, blaVIM-35, blaVIM-37, blaVIM-39, blaVIM-4, blaVIM-42, blaVIM-43, blaVIM-10, blaVIM-11, blaVIM-15, blaVIM-16, blaVIM-17, blaVIM-23, blaVIM-24, blaVIM-30, blaVIM-31, blaVIM-8, blaVIM-9, blaVIM-18, blaVIM-3, blaVIM-36, blaVIM-6, blaVIM-25, blaVIM-38, blaVIM-5

[a] Results include combinations of AMR gene variants and organisms tested in the study that are absent from Table 1.
[b] One gene variant of aadA7 (NCBI Accession AF224733) is not consistently detected by the Acuitas AMR Gene Panel due to 4 mismatches with the reverse PCR primer and one mismatch with the PCR probe of the AAD assay. Other aadA7 variants lack the mismatches and are expected to be consistently detected.
[c] Only these two Sul3 sequences are predicted to be detected by the Acuitas AMR Gene Panel. Other Sul3 accession numbers are not predicted to be detected.
[d] Detectable indicates 100% homology of each primer/detector probe(s) with the target sequence. Likely Detectable indicates <100% homology of one or both primers with the target sequence (one mismatch in one or both primers) and 100% homology of detector probe(s). Potentially Detectable indicates <95% homology of one or more primers with the target sequence but ≤2 nucleotide mismatches over their entire length along with 100% homology of detector probe(s).
[e] The test panel for this study was composed of 5 *Enterococcus faecalis*, 69 *Escherichia coli*, 3 *Klebsiella pneumoniae ssp ozaenae*, 89 *Klebsiella pneumoniae ssp pneumoniae*, 34 *Proteus mirabilis*, and 98 *Pseudomonas aeruginosa strains*.

3. Analytical Specificity (Cross-Reactivity)

The Acuitas AMR Gene Panel was investigated for potential cross-reactivity with AMR genes. Cross-reactivity was also evaluated for the test's species identification assays for *E. coli* and *P. aeruginosa*, which are used in conjunction with mutant gyrase assays for these two organisms.

Analytical specificity (cross-reactivity) of the Acuitas AMR Gene Panel was evaluated for a total of four hundred and twenty-three (423) isolates. One hundred twenty-five (125) isolates were tested in duplicate from a single 0.5 McFarland bacterial suspension to evaluate analytical specificity at the organism and AMR gene level. Additionally, cross-reactivity at the AMR gene level was evaluated for two-hundred and ninety-eight (298) isolates from the Analytical Reactivity (Inclusivity) Study.

Acuitas AMR Gene Panel results were compared with species identification by well-established automated species identification methods and AMR gene detection by Whole Genome Sequencing.

Organisms that did not cross-react with the *E. coli* or *P. aeruginosa* species identification assays of the Acuitas AMR Gene Panel are provided in *Table 6 - Organisms without Cross-Reactivity*.

Table 6 - Organisms without Cross-Reactivity

Bacterial Species		
Achromobacter xylosoxidans	*Enterococcus gallinarum*	*Raoultella planticola*
Acinetobacter baumannii complex	*Enterococcus gilvus*	*Salmonella enterica*
Acinetobacter ursingii	*Enterococcus hirae*[c]	*Salmonella species*
Aeromonas hydrophila	*Enterococcus italicus*	*Serratia marcescens*
Candida albicans	*Enterococcus pseudoavium*	*Serratia plymuthica*
Citrobacter braakii	*Enterococcus raffinosus*	*Shigella boydii*[d]
Citrobacter freundii complex	*Hafnia alvei*	*Shigella dysenteriae*[d]
Citrobacter koseri	*Klebsiella oxytoca*	*Sphingomonas paucimobilis*
Citrobacter youngae	*Klebsiella oxytoca ESBL*	*Staphylococcus aureus*
Clostridioides (Clostridium) difficile	*Leclercia adecarboxylata*	*Staphylococcus capitis*
Corynebacterium diphtheriae	*Moraxella catarrhalis*[c]	*Staphylococcus epidermidis*
Escherichia fergusonii[a]	*Morganella morganii* ssp *morganii*	*Staphylococcus haemolyticus*
Escherichia hermannii[b]	*Proteus vulgaris*	*Staphylococcus hominis*
Enterobacter aerogenes	*Providencia rettgeri*	*Staphylococcus lugdunensis*
Enterobacter cloacae	*Providencia stuartii*	*Staphylococcus saprophyticus*
Enterobacter cloacae complex	*Pseudomonas fluorescens*	*Staphylococcus warneri*
Enterobacter hormaechei[c]	*Pseudomonas luteola*	*Streptococcus agalactiae*

Bacterial Species		
Enterococcus dispar[c]	*Pseudomonas oryzihabitans*	*Streptococcus pyogenes*
Enterococcus durans	*Pseudomonas putida*	*Yersinia pseudotuberculosis*
Enterococcus faecium	*Pseudomonas stutzeri*	

[a.] One isolate (Parent LDW L00021948-001) was identified as *Escherichia fergusonii* by ATCC and by WGS whereas the automated species identification method reported it as *E. coli*. The Acuitas AMR Gene Panel reported true negative for *P. aeruginosa* species ID and false negative for *E. coli* species ID with this isolate based on the automated species identification method designation as *E. coli.* This isolate is included as *E. fergusonii* based on ATCC and WGS designation.

[b.] One isolate (Parent LDW L00017841-001) was identified as *Escherichia hermannii* by ATCC and as *Enterobacterales* by WGS. The automated species identification method showed the isolate as *E. coli*. The Acuitas AMR Gene Panel reported true negative for *P. aeruginosa* species ID and false negative for *E. coli* species ID with this isolate based on the automated species identification method designation as *E. coli*. This isolate is included as *E. hermannii* based on ATCC and WGS designation.

[c.] These isolates are included based on species designations by ATCC and/or WGS.

[d.] Some but not all isolates of *Shigella boydii* and *Shigella dysenteriae* cross-react with the *E. coli* species ID assay of the Acuitas AMR Gene Panel.

Isolates demonstrating cross-reactivity with the species identification assays of the Acuitas AMR Gene Panel are summarized in *Table 7 - Organisms Demonstrating Cross-Reactivity*.

Table 7 - Organisms Demonstrating Cross-Reactivity

External Strain ID	Organism	Positive Species Identification Assay
AR-0030	*Shigella sonnei*	E. coli ID
ATCC 8700	*Shigella boydii*	E. coli ID
ATCC 35966	*Shigella boydii*	E. coli ID
99-10354	*Shigella dysenteriae*	E. coli ID
ATCC 9361	*Shigella dysenteriae*	E. coli ID
ATCC 12022	*Shigella flexneri*	E. coli ID
ATCC 9199	*Shigella flexneri*	E. coli ID
ATCC 12025	*Shigella flexneri*	E. coli ID
ATCC 9290	*Shigella sonnei*	E. coli ID

Shigella boydii, Shigella dysenteriae, Shigella flexneri, and *Shigella sonnei* cross-reacted with the *E. coli* ID assay on the Acuitas AMR Gene Panel as expected from *in silico* analysis, although one (1) isolate of *Shigella boydii* (Parent LDW L00021962-001, External ID ATCC 9207) and one (1) isolate of *Shigella dysenteriae* (Parent LDW L00021966-001, External ID ATCC 49347) did not cross-react with the *E. coli* ID target. All *Shigella* strains that demonstrated cross-reactivity with the *E. coli* ID assay target had wild-type gyrase sequences. Therefore, no determination could be made regarding cross-reactivity of *Shigella* species with the *E. coli gyr*A Mutant assay.

No cross-reactivity was observed with genes/variants not targeted by the Acuitas AMR Gene Panel. *Table 8 - AMR Gene Variants without Cross-Reactivity* summarizes AMR genes that did not cross-react with the Acuitas AMR Gene Panel. The genes (or variants) in *Table 8* were identified in a cross-reactivity panel of organisms by WGS and are not specific targets of the Acuitas AMR Gene Panel.

Table 8 - AMR Gene Variants without Cross-Reactivity

AMR Gene Variants					
aac(2')-Ia	aph(3')-IIa	blaMIR-5	cphA2	mcr-3	QnrVC1
aac(3)-Ia	aph(3')-IIb	blaMOX-3	dfrA1	mecA	rmtC
aac(3)-Ic	aph(3')-VIa	blaOXA-10	dfrA12	mph(A)	rmtD
aac(3)-Id	aph(3')-VIb	blaOXA-114	dfrA14	mph(C)	spc
aac(6')	aph(3')-XV	blaOXA-17	dfrA15	mph(D)	strA
aac(6')-31	aph(6)-Ic	blaOXA-2	dfrA18	mph(E)	strB
aac(6')-33	ARR-2	blaOXA-5	dfrA23	msr(A)	Sul3[a]
aac(6')-aph(2'')	ARR-3	blaOXA-50	dfrA27	msr(C)	tet(41)
aac(6')-Ic	blaACC-1	blaOXA-56	dfrA29	msr(E)	tet(A)
aac(6')-Il	blaACT-12	blaOXA-94	dfrA30	norA	tet(B)
aac(6')-IIc	blaACT-16	blaOXY-1	dfrA31	oqxA	tet(C)
aac(6')-Im	blaACT-25	blaOXY-2	dfrA32	oqxB	tet(D)
aac(6')-Iq	blaACT-27	blaPAO	dfrA3b	qepA	tet(G)
aacA29	blaACT-28	blaSME-4	dfrB1	QnrA1	tet(H)
aadA11	blaACT-35	blaZ	dfrB5	QnrB1	tet(J)
aadA14	blaACT-37	cat	dfrG	QnrB12	tet(K)
aadA16	blaACT-7	cat(pC221)	ere(A)	QnrB19	tet(M)
aadA24	blaADC-25	catA1	ere(B)	QnrB2	tet(X)
aadA5	blaCARB-2	catA2	erm(42)	QnrB34	VanH-A
aadA6	blaCMY-100	catA3	erm(A)	QnrB4	VanR-A
aadA7[b]	blaHERA-1	catB10	erm(B)	QnrB6	VanS-A
aadD	blaLEN11	catB3	erm(C)	QnrB68	VanX-A
ampH	blaLEN17	catB7	floR	QnrB7	VanY-A
ant(6)-Ia	blaLEN22	catB8	fosA	QnrB88	VanZ-A
aph(2'')-Ib	blaLUT-1	cml	hugA	QnrD	
aph(3')-Ia	blaMAL-1	cmlA1	lnu(F)	QnrS1	

AMR Gene Variants					
aph(3')-Ic	blaMIR-3	cmx	Isa(A)	QnrS3	

[a.] Sul3 sequences in NCBI other than accession numbers AY047357 and AB281183.
[b.] aadA7 is not consistently detected by the AMR Gene Panel due to 4 mismatches for the reverse PCR primer and one mismatch for the PCR probe of the AAD assay.

4. Fresh versus Frozen

The equivalence of fresh bacterial isolates and bacterial suspensions, previously prepared and stored frozen at -20 °C, was established in a Fresh versus Frozen study to assess the performance of both preparations on the Acuitas AMR Gene Panel. A panel of nine (9) isolates covering all detected species and majority of the antimicrobial resistance genes in the Acuitas AMR Gene Panel were tested as a part of this study; refer to *Table 9 - Fresh vs. Frozen Test Panel* for the complete fresh versus frozen test panel. Each of the study isolates was cultured on blood agar from which 0.5 McFarland suspensions were prepared and aliquoted. 500 µL 0.5 McFarland aliquots were prepared for each isolate and each positive and negative external control. Positive and negatives controls prepared on Day 1 of T=0 testing were used throughout the duration of study.

Immediately following preparation of the 0.5 McFarland suspensions, ten (10) aliquots of each isolate were extracted for performing the T=0 testing event; this data served as the control data set. The remaining aliquots for each isolate, positive control, and negative control were stored at -20 °C until the final T=56 days testing event. Storage conditions are summarized below in *Table 10 - Fresh vs. Frozen Study Storage Conditions*.

Ten (10) replicates of each isolate were evaluated at each of two testing events (i.e., T=0, T=56). Testing for each timepoint was split across three (3) days, with each of three (3) operators testing ten (10) replicates of a single isolate, one (1) positive control and one (1) negative control on each day of testing.

Table 9 - Fresh vs. Frozen Test Panel

Parent LDW Number/External ID	Study LDW Number	Organism	AMR Genes Identified by Whole Genome Sequencing
L00000068-001	L00022847-001	*E. coli*	ANT, DFR, *E. coli gyr*A Mutant, KPC, Sul1, Sul2, TEM
L00015886-001	L00022848-001	*E. coli*	Sul2, TEM
L00009154-001[a]	L00022849-001	*E. coli*	DFR, Sul1, Sul2, TEM
L00009721-001	L00022850-001	*K. pneumoniae*	AAC, AAD, CMY, CTX-M-1, NDM, OXA-48, Sul1, Sul2, TEM
L00007800-001	L00022851-001	*K. pneumoniae*	AAC, CTX-M-1, IMP, OXA-1, Sul1, Sul2, TEM
L00008624-001	L00022852-001	*P. aeruginosa*	*P. aeruginosa gyr*A Mutant, PER, VIM
L00013504-001	L00022853-001	*P. mirabilis*	AAC, ANT, CMY, Sul2, TEM, VEB
L00013200-001[b]	L00022854-001	*P. mirabilis*	AAC, APH, CTX-M-9, DFR, OXA-1, Sul2, TEM

Parent LDW Number/External ID	Study LDW Number	Organism	AMR Genes Identified by Whole Genome Sequencing
ATTC BAA-2573	L00022855-001	*E. faecalis*	vanA

[a.] WGS results compiled from different sets of AMR Gene and Gyrase data.
[b.] WGS results compiled from merged sets of AMR Gene data.

Prepared aliquots 0.5 McFarland suspensions, described above, of isolates as well as positive and negative controls were stored under conditions summarized in *Table 10 - Fresh vs. Frozen Study Storage Conditions* and tested with the Acuitas AMR Gene Panel.

Table 10 - Fresh vs. Frozen Study Storage Conditions

Specimen Type	Storage Conditions	Number Isolates Tested	Replicates per Isolate
Fresh 0.5 McFarland	T = 0 days	9	10
Frozen 0.5 McFarland	T = 56 days at -15 to -25 °C	9	10

Overall, 100% agreement was observed between the T=0 and frozen T=56 time points as summarized in *Table 11 - Fresh versus Frozen Results*.

Table 11 - Fresh versus Frozen Results

Study LDW Number	Organism	Testing Event	Replicates per Isolate	Replicates Matching Expected Results
L00022847-001	*E. coli*	T=0	10	100%
		T=56	10	100%
L00022848-001	*E. coli*	T=0	10	100%
		T=56	10	100%
L00022849-001	*E. coli*	T=0	10	100%
		T=56	10	100%
L00022850-001	*K. pneumoniae*	T=0	10	100%
		T=56	10	100%
L00022851-001	*K. pneumoniae*	T=0	10	100%
		T=56	10	100%
L00022852-001	*P. aeruginosa*	T=0	10	100%
		T=56	10	90%[a]
L00022853-001	*P. mirabilis*	T=0	10	90%[b]

Study LDW Number	Organism	Testing Event	Replicates per Isolate	Replicates Matching Expected Results
		T=56	10	100%
L00022854-001	*P. mirabilis*	T=0	10	100%
		T=56	10	100%
L00022855-001	*E. faecalis*	T=0	10	100%
		T=56	10	100%

[a.] One (1) of ten (10) replicates of isolate L00022852-001 at T=56 had a false positive for ANT gene target. The time point for isolate L00022852-001 was repeated, and all ten (10) replicates returned expected gene targets. Substituting original results with retest results would improve the NPA to 100%.

[b.] One of ten (10) replicates of L00022853-001 (T=0) had a false positive for DFR (dfrA17 gene). The time point for isolate L00022853-001 was repeated, and all ten (10) replicates returned expected gene targets. Substituting original results with retest results would improve the NPA to 100%.

5. Sample Stability - Extracted DNA

The performance of the Acuitas AMR Gene Panel was evaluated using extracted DNA stored at 15-25 °C and/or 2-8 °C prior to testing. Five (5) isolates harboring most of the AMR genes detected by the Acuitas AMR Gene Panel (*Table 12 - Extracted DNA Stability Test Panel*) were tested by one operator across two (2) OpGen Qualified QuantStudio 5 instruments. Three (3) replicates of each isolate were evaluated for each testing event.

Table 12 - Extracted DNA Stability Test Panel

Parent LDW Number/External ID	Study LDW Number	Organism	AMR Genes Identified by Whole Genome Sequencing
L00005419-001	L00021522-001	*E. coli*	AAC, CTX-M-9, DFR, *E. coli gyr*A Mutant, Sul1, Sul2, TEM
L00003125-002	L00021523-001	*K. pneumoniae*	AAC, AAD, DHA, NDM, Sul1, Sul2
L00000353-001	L00021524-001	*P. aeruginosa*	None
L00012247-001	L00021525-001	*P. mirabilis*	AAC, CTX-M-2, Sul2, TEM
L00021516-001/ FS10	L00021521-001	*E. faecalis*	None

The isolates were extracted and stored under the conditions specified in *Table 13 - Extracted DNA Storage Conditions* before testing at OpGen by a single operator on two (2) QuantStudio5 instruments.

Table 13 - Extracted DNA Storage Conditions

Storage Conditions	Number of Isolates Tested	Replicates per Isolate
T = 0	5	3

Storage Conditions	Number of Isolates Tested	Replicates per Isolate
T = 6 hours at 15-25 °C	5	3
T = 6 hours at 15-25 °C, then 7 days at 2-8 °C	5	3
T = 7 days at 2-8 °C	5	3

The five (5) isolates were cultured on blood agar. Fourteen (14) 0.5 McFarland suspensions per isolate were prepared and DNA extracted. DNA extracts per isolate were pooled and aliquoted (500 µL) for storage (*Table 13)*. Three (3) 150µL replicates of extracted DNA were evaluated per isolate per storage condition.

Overall agreement between T=0 and storage conditions are summarized in *Table 14 - Extracted DNA Stability Results*. There was 100% agreement for all samples and storage conditions compared with the T=0 timepoint, supporting the claim that DNA extracted on the QIAGEN EZ1 Advanced XL System can be stored as follows before testing on the OpGen Qualified QuantStudio 5 instrument:

- Up to six (6) hours at room temperature;
- Up to seven (7) days at 2-8 °C;
- Up to six (6) hours at room temperature followed by up to seven (7) days at 2-8 °C.

Table 14 - Extracted DNA Stability Results

Study LDW Number (Organism)	Testing Event	Replicates Tested	Replicates Matching T=0 Results (%)
L00021521-001 (*E. faecalis*)	T = 6 hours at 15-25 °C	3	3/3 (100%)
	T = 6 hours at 15-25 °C, then 7 days at 2-8 °C	3	3/3 (100%)
	T = 7 days at 2-8 °C	3	3/3 (100%)
L00021522-001 (*E. coli*)	T = 6 hours at 15-25 °C	3	3/3 (100%)
	T = 6 hours at 15-25 °C, then 7 days at 2-8 °C	3	3/3 (100%)
	T = 7 days at 2-8 °C	3	3/3 (100%)
L00021523-001 (*K. pneumoniae*)	T = 6 hours at 15-25 °C	3	3/3 (100%)
	T = 6 hours at 15-25 °C, then 7 days at 2-8 °C	3	3/3 (100%)
	T = 7 days at 2-8 °C	3	3/3 (100%)
L00021524-001 (*P. aeruginosa*)	T = 6 hours at 15-25 °C	3	3/3 (100%)
	T = 6 hours at 15-25 °C, then 7 days at 2-8 °C	3	3/3 (100%)
	T = 7 days at 2-8 °C	3	3/3 (100%)

Study LDW Number (Organism)	Testing Event	Replicates Tested	Replicates Matching T=0 Results (%)
L00021525-001 (*P. mirabilis*)	T = 6 hours at 15-25 °C	3	3/3 (100%)
	T = 6 hours at 15-25 °C, then 7 days at 2-8 °C	3	3/3 (100%)
	T = 7 days at 2-8 °C	3	3/3 (100%)

6. Sample Stability - Frozen DNA

The performance of the Acuitas AMR Gene Panel was also evaluated using extracted DNA stored up to 30 days at -15 °C to -25 °C. Five (5) isolates harboring most of the AMR genes detected by the Acuitas AMR Gene Panel (*Table 15 - Frozen DNA Test Panel*) were tested by one (1) operator across two (2) OpGen Qualified QuantStudio 5 instruments. Three (3) replicates of each isolate were evaluated for each testing event.

Table 15 - Frozen DNA Test Panel

Parent LDW Number/External ID	Study LDW Number	Organism	AMR Genes Identified by Whole Genome Sequencing
L00005419-001	L00021936-001	*E. coli*	AAC, CTX-M-9, DFR, *E. coli gyr*A Mutant, Sul1, Sul2, TEM
L00003125-002	L00021937-001	*K. pneumoniae*	AAC, AAD, DHA, NDM, Sul1, Sul2
L00000353-001	L00021938-001	*P. aeruginosa*	None
L00012247-001	L00021939-001	*P. mirabilis*	AAC, CTX-M-2, Sul2, TEM
L00021516-001/ FS10	L00021940-001	*E. faecalis*	None

Fifteen (15) 0.5 McFarland suspensions were prepared per isolate and DNA extracted. DNA extracts per each isolate were pooled and aliquoted into unique 150 µL aliquots. Three (3) DNA aliquots per isolate were tested immediately (T=0). Remaining aliquots were stored at -15 to -25 °C for 14 or 30 days before testing.

Overall agreement was 100% between the T=0 timepoint and tested storage conditions (*Table 16 - Frozen DNA Results*).

Table 16 - Frozen DNA Results

Study LDW Number	Timepoint	Replicates Tested	Replicates Matching Expected Results (%)
L00021936-001 *(E. coli)*	T=14 days at -20 °C	3	3/3 (100%)
	T=30 days at -20 °C	3	3/3 (100%)
L00021937-001	T=14 days at -20 °C	3	3/3 (100%)

Study LDW Number	Timepoint	Replicates Tested	Replicates Matching Expected Results (%)
(K. pneumoniae)	T=30 days at -20 °C	3	3/3 (100%)
L00021938-001 *(P. aeruginosa)*	T=14 days at -20 °C	3	3/3 (100%)
	T=30 days at -20 °C	3	3/3 (100%)
L00021939-001 *(P. mirabilis)*	T=14 days at -20 °C	3	3/3 (100%)
	T=30 days at -20 °C	3	3/3 (100%)
L00021940-001 *(E. faecalis)*	T=14 days at -20 °C	3	3/3 (100%)
	T=30 days at -20 °C	3	3/3 (100%)

7. Sample Stability - Plated DNA

This study evaluated the stability of prepared Acuitas AMR Gene Panel plates, containing extracted DNA and Master Mix, after storage for 6 hours at 2-8 °C. Five (5) isolates harboring most of the AMR genes detected by the Acuitas AMR Gene Panel (*Table 17 - Prepared Plate Test Panel*) were tested by one (1) operator across two (2) OpGen Qualified QuantStudio 5 instruments. Three (3) replicates of each isolate were evaluated for each testing event.

Table 17 - Prepared Plate Test Panel

Original LDW Number/External ID	LDW Number for Study	Organism	AMR Genes Identified by Whole Genome Sequencing
L00005419-001	L00021931-001	*E. coli*	AAC, CTX-M-9, DFR, *E. coli gyr*A Mutant, Sul1, Sul2, TEM
L00003125-002	L00021932-001	*K. pneumoniae*	AAC, AAD, DHA, NDM, Sul1, Sul2
L00000353-001	L00021933-001	*P. aeruginosa*	None
L00012247-001	L00021934-001	*P. mirabilis*	AAC, CTX-M-2, Sul2, TEM
L00021516-001/ FS10	L00021935-001	*E. faecalis*	None

Seven (7) 0.5 McFarland suspensions were prepared and extracted per isolate. Extracts per isolate were pooled and aliquoted (500 µL). Extracted DNA samples (150 µL) were combined with Acuitas AMR Gene Panel Master Mix (CP3402) and added to Acuitas AMR Gene Panel assay plates (CP3230). Prepared plates were stored for zero or 6 hours at 2-8 °C (*Table 18 - Prepared Plate Storage Conditions*) and tested.

Table 18 - Prepared Plate Storage Conditions

Storage Condition	Number of Isolates Tested	Replicates per Isolate
T = 0	5	3

T = 6 hours at 2-8 °C	5	3

Overall agreement was 100% between T=0 and T=6 hours at 2-8 °C (*Table 19 - Prepared Plate Results*).

Table 19 - Prepared Plate Results

Study LDW Number (Organism)	Timepoint	Replicates Tested	Replicates matching T=0 Results (%)
L00021931-001 (*E. coli*)	T = 6 hours, 2-8 °C	3	3/3 (100%)
L00021932-001 (*K. pneumoniae*)	T = 6 hours, 2-8 °C	3	3/3 (100%)
L00021933-001 (*P. aeruginosa*)	T = 6 hours, 2-8 °C	3	3/3 (100%)
L00021934-001 (*P. mirabilis*)	T = 6 hours, 2-8 °C	3	3/3 (100%)
L00021935-001 (*E. faecalis*)	T = 6 hours, 2-8 °C	3	3/3 (100%)

8. Media Equivalency

The equivalence of blood agar and MacConkey agar media when used to culture pure bacterial colonies for use with the Acuitas AMR Gene Panel test was established by evaluating the performance of fifty-two (52) isolates harboring most of the AMR genes detected by the Acuitas AMR Gene Panel (*Table 20 - Media Equivalency Test Panel*).

Table 20 - Media Equivalency Test Panel

Organism	Species ID and AMR Genes Detected by Whole Genome Sequencing	Study LDW Number	Parent LDW Number
E. coli	AAC, CMY, CTX-M-1, DFR, *E. coli gyr*A Mutant, OXA-1, SHV, Sul1, Sul2	L00021535-001	L00009217-001
E. coli	AAC, ANT, CTX-M-1, *E. coli gyr*A Mutant, Sul1, Sul2, TEM	L00021536-001	L00008878-001
E. coli	None	L00021537-001	L00015883-001
E. coli	Sul2	L00021538-001	L00015885-001
E. coli	DFR, Sul2, TEM	L00021539-001	L00009368-001
E. coli	ANT, *E. coli gyr*A Mutant, KPC, SHV, TEM	L00021540-001	L00009104-001
E. coli	AAC, DFR, Sul1, Sul2, TEM	L00021541-001	L00009121-001
E. coli	CMY, CTX-M-1, *E. coli gyr*A Mutant, Sul2, TEM	L00021542-001	L00008677-001
E. coli	KPC, TEM	L00021543-001	L00009056-001
E. coli	None	L00021544-001	L00015917-001

Organism	Species ID and AMR Genes Detected by Whole Genome Sequencing	Study LDW Number	Parent LDW Number
E. coli	AAC, Sul2, TEM	L00021575-001	L00014163-001
E. coli	AAC, CTX-M-1, DFR, *E. coli gyr*A Mutant, OXA-1, Sul1, Sul2	L00021576-001	L00015876-001
E. coli	None	L00021577-001	L00015883-001
K. pneumoniae	AAC, AAD, CTX-M-1, DFR, KPC, NDM, Sul1, TEM	L00021545-001	L00005469-001
K. pneumoniae	AAC, AAD, APH, CTX-M-1, OXA-1, OXA-48, Sul1, TEM	L00021546-001	L00008681-001
K. pneumoniae	None	L00021547-001	L00015913-001
K. pneumoniae	None	L00021548-001	L00015918-001
K. pneumoniae	AAC, KPC, Sul1, TEM	L00021549-001	L00009602-001
K. pneumoniae	CTX-M-1, RMT, Sul2, TEM	L00021550-001	L00011112-001
K. pneumoniae	AAC, AAD, KPC, OXA-9, TEM	L00021551-001	L00007709-001
K. pneumoniae	AAC, CTX-M-1, Sul2, TEM	L00021552-001	L00007888-001
K. pneumoniae	AAC, CTX-M-1, OXA-1, RMT, Sul1	L00021553-001	L00009765-001
K. pneumoniae	None	L00021554-001	L00000120-001
K. pneumoniae	None	L00021578-001	L00015918-001
K. pneumoniae	AAC, AAD, CTX-M-1, OXA-1, OXA-48, Sul1	L00021579-001	L00015736-001
K. pneumoniae	AAC, CTX-M-1, OXA-1, OXA-48	L00021580-001	L00015867-001
P. aeruginosa	AAC, ANT, OXA-1, *P. aeruginosa gyr*A Mutant, VIM	L00021555-001	L00008639-001
P. aeruginosa	AAC, *P. aeruginosa gyr*A Mutant	L00021556-001	L00004931-001
P. aeruginosa	None	L00021557-001	L00015786-001
P. aeruginosa	AAC, *P. aeruginosa gyr*A Mutant	L00021558-001	L00015788-001
P. aeruginosa	None	L00021559-001	L00015789-001
P. aeruginosa	None	L00021560-001	L00015790-001
P. aeruginosa	AAC, *P. aeruginosa gyr*A Mutant	L00021561-001	L00010360-001
P. aeruginosa	AAC, CTX-M-1, OXA-1, TEM[a]	L00021562-001	L00008666-001
P. aeruginosa	None	L00021563-001	L00015792-001
P. aeruginosa	*P. aeruginosa gyr*A Mutant, VIM	L00021564-001	L00009440-001

Organism	Species ID and AMR Genes Detected by Whole Genome Sequencing	Study LDW Number	Parent LDW Number
P. aeruginosa	None	L00021581-001	L00010871-001[b]
P. aeruginosa	AAC, *P. aeruginosa gyr*A Mutant, VIM	L00021582-001	L00015582-001
P. aeruginosa	AAC, *P. aeruginosa gyr*A Mutant, VIM	L00021583-001	L00015586-001
P. mirabilis	AAC, APH, CTX-M-9, DFR, OXA-1, Sul2, TEM	L00021565-001	L00012352-001
P. mirabilis	Sul2, TEM	L00021566-001	L00015759-001
P. mirabilis	AAC, ANT, CTX-M-9, DFR, Sul2, TEM	L00021567-001	L00012613-001
P. mirabilis	None	L00021568-001	L00015801-001
P. mirabilis	AAC, armA, CMY, TEM	L00021569-001	L00012786-001
P. mirabilis	AAC, APH, OXA-1, Sul2	L00021570-001	L00015806-001
P. mirabilis	AAC, CTX-M-2, OXA-9, Sul2, TEM	L00021571-001	L00012812-001
P. mirabilis	None	L00021572-001	L00015828-001
P. mirabilis	CMY, NDM, Sul2	L00021573-001	L00012564-001
P. mirabilis	None	L00021574-001	L00015912-001
P. mirabilis	None	L00021584-001	L00013441-001
P. mirabilis	TEM	L00021585-001	L00015645-001
P. mirabilis	None	L00021586-001	L00015912-001

[a.] Re-sequencing of isolate L00021562-001 unambiguously identified the species as *P. aeruginosa* and detected AMR gene variants *P. aeruginosa gyr*A Mutant and VEB.
[b.] WGS results compiled from different sets of AMR gene and Gyrase data.

Thirteen (13) isolates per organism (*E. coli, K. pneumoniae, P. aeruginosa,* and *P. mirabilis*) were cultured on both blood agar and MacConkey agar. *E. faecalis* was not tested as it does not grow on MacConkey agar. A 0.5 McFarland suspension was prepared from pure colonies on the blood and MacConkey agar for testing with the Acuitas AMR Gene Panel.

Percent agreement was determined for detection of AMR genes by the Acuitas AMR Gene Panel versus Whole Genome Sequencing for each isolate replicate from blood and MacConkey agar.

Acuitas AMR Gene Panel results were compiled and analyzed to show percent agreement with the two comparator methods (well-established automated species identification methods and Whole Genome Sequencing) for each combination of organism, AMR gene and agar media, as summarized in *Table 21 - Performance from MacConkey and Blood Agar*, which represents original results without inclusion or analysis of repeat results. Isolate (testing event) agreement across all AMR genes and both types of agar media ranged from 62 to 92% across the four organisms versus the two comparator

methods (not shown directly in Table 23). Incorporation of repeat test results as described in the footnotes of *Table 21* would improve isolate agreement as follows: *E. coli* on blood (100%), *E. coli* on MacConkey (100%), *K. pneumoniae* on blood (100%), *K. pneumoniae* on MacConkey (100%), *P. aeruginosa* on blood (100%), *P. aeruginosa* on MacConkey (100%), *P. mirabilis* on blood (92%), and *P. mirabilis* on MacConkey (92%).

The study did not uncover evidence of a media effect between blood and MacConkey agar, determining that both are suitable for the Acuitas AMR Gene Panel.

Table 21 - Performance from MacConkey and Blood Agar

Organism	Resistance Marker	Number of Testing Events	Blood Agar		MacConkey Agar	
			PPA (TP/(TP+FN)) (95% CI)	NPA (TN/(TN+FP)) (95% CI)	PPA (TP/(TP+FN)) (95% CI)	NPA (TN/(TN+FP)) (95% CI)
E. coli	AAC	13	100% (7/7) (64.57 - 100)	100% (6/6) (60.97 - 100)	100% (7/7) (64.57 - 100)	100% (6/6) (60.97 - 100)
	ANT	13	100% (2/2) (34.24 - 100)	100% (11/11) (74.12 - 100)	100% (2/2) (34.24 - 100)	100% (11/11) (74.12 - 100)
	CMY	13	100% (2/2) (34.24 - 100)	100% (11/11) (74.12 - 100)	100% (2/2) (34.24 - 100)	100% (11/11) (74.12 - 100)
	CTX-M-1	13	100% (6/6) (60.97 - 100)	100% (7/7) (64.57 - 100)	100% (6/6) (60.97 - 100)	100% (7/7) (64.57 - 100)
	CTX-M-2	13	-	100% (13/13) (77.19 - 100)	-	100% (13/13) (77.19 - 100)
	CTX-M-9	13	-	100% (13/13) (77.19 - 100)	-	100% (13/13) (77.19 - 100)
	DFR	13	100% (4/4) (51.01 - 100)	100% (9/9) (70.08 - 100)	100% (4/4) (51.01 - 100)	100% (9/9) (70.08 - 100)
	E. coli gyrA	13	100% (7/7) (64.57 - 100)	100% (6/6) (60.97 - 100)	100% (7/7) (64.57 - 100)	100% (6/6) (60.97 - 100)
	KPC	13	100% (2/2) (34.24 - 100)	100% (11/11) (74.12 - 100)	100% (2/2) (34.24 - 100)	100% (11/11) (74.12 - 100)
	MCR-1	13	-	100% (13/13) (77.19 - 100)	-	100% (13/13) (77.19 - 100)
	OXA-1	13	100% (4/4) (51.01 - 100)	100% (9/9) (70.08 - 100)	100% (4/4) (51.01 - 100)	100% (9/9) (70.08 - 100)
	OXA-9	13	-	100% (13/13) (77.19 - 100)	-	100% (13/13) (77.19 - 100)
	SHV	13	100% (2/2) (34.24 - 100)	100% (11/11) (74.12 - 100)	100% (2/2) (34.24 - 100)	100% (11/11) (74.12 - 100)
	Sul1	13	100% (4/4) (51.01 - 100)	100% (9/9) (70.08 - 100)	100% (4/4) (51.01 - 100)	100% (9/9) (70.08 - 100)
	Sul2	13	100% (8/8) (67.56 - 100)	100% (5/5) (56.55 - 100)	100% (8/8) (67.56 - 100)	100% (5/5) (56.55 - 100)
	TEM	13	100% (9/9) (70.08 - 100)	100% (4/4) (51.01 - 100)	100% (9/9) (70.08 - 100)	100% (4/4) (51.01 - 100)
K. pneumoniae	AAC	13	100% (8/8) (67.56 - 100)	100% (5/5) (56.55 - 100)	100% (8/8) (67.56 - 100)	100% (5/5) (56.55 - 100)

Organism	Resistance Marker	Number of Testing Events	Blood Agar		MacConkey Agar	
			PPA (TP/(TP+FN)) (95% CI)	NPA (TN/(TN+FP)) (95% CI)	PPA (TP/(TP+FN)) (95% CI)	NPA (TN/(TN+FP)) (95% CI)
	AAD [a]	13	100% (4/4) (51.01 - 100)	100% (9/9) (70.08 - 100)	75% (3/4) (30.06 - 95.44)	100% (9/9) (70.08 - 100)
	APH	13	100% (1/1) (20.65 - 100)	100% (12/12) (75.75 - 100)	100% (1/1) (20.65 - 100)	100% (12/12) (75.75 - 100)
	CMY	13	-	100% (13/13) (77.19 - 100)	-	100% (13/13) (77.19 - 100)
	CTX-M-1 [b]	13	85.7% (6/7) (48.69 - 97.43)	100% (6/6) (60.97 - 100)	100% (7/7) (64.57 - 100)	100% (6/6) (60.97 - 100)
	CTX-M-9	13	-	100% (13/13) (77.19 - 100)	-	100% (13/13) (77.19 - 100)
	DFR	13	100% (1/1) (20.65 - 100)	100% (12/12) (75.75 - 100)	100% (1/1) (20.65 - 100)	100% (12/12) (75.75 - 100)
	DHA	13	-	100% (13/13) (77.19 - 100)	-	100% (13/13) (77.19 - 100)
	IMP	13	-	100% (13/13) (77.19 - 100)	-	100% (13/13) (77.19 - 100)
	KPC	13	100% (3/3) (43.85 - 100)	100% (10/10) (72.25 - 100)	100% (3/3) (43.85 - 100)	100% (10/10) (72.25 - 100)
	NDM	13	100% (1/1) (20.65 - 100)	100% (12/12) (75.75 - 100)	100% (1/1) (20.65 - 100)	100% (12/12) (75.75 - 100)
	OXA-1	13	100% (4/4) (51.01 - 100)	100% (9/9) (70.08 - 100)	100% (4/4) (51.01 - 100)	100% (9/9) (70.08 - 100)
	OXA-9	13	100% (1/1) (20.65 - 100)	100% (12/12) (75.75 - 100)	100% (1/1) (20.65 - 100)	100% (12/12) (75.75 - 100)
	OXA-48	13	100% (3/3) (43.85 - 100)	100% (10/10) (72.25 - 100)	100% (3/3) (43.85 - 100)	100% (10/10) (72.25 - 100)
	RMT [b]	13	50% (1/2) (9.45 - 90.55)	100% (11/11) (74.12 - 100)	100% (2/2) (34.24 - 100)	100% (11/11) (74.12 - 100)
	Sul1 [a]	13	100% (5/5) (56.55 - 100)	100% (8/8) (67.56 - 100)	80% (4/5) (37.55 - 96.38)	100% (8/8) (67.56 - 100)
	Sul2	13	100% (2/2) (34.24 - 100)	100% (11/11) (74.12 - 100)	100% (2/2) (34.24 - 100)	100% (11/11) (74.12 - 100)
	TEM [b]	13	83.3% (5/6) (43.65 - 96.99)	100% (7/7) (64.57 - 100)	100% (6/6) (60.97 - 100)	100% (7/7) (64.57 - 100)
P. aeruginosa	AAC	13	85.7% (6/7) (48.69 - 97.43)	100% (6/6) (60.97 - 100)	85.7% (6/7) (48.69 - 97.43)	100% (6/6) (60.97 - 100)

Organism	Resistance Marker	Number of Testing Events	Blood Agar		MacConkey Agar	
			PPA (TP/(TP+FN)) (95% CI)	NPA (TN/(TN+FP)) (95% CI)	PPA (TP/(TP+FN)) (95% CI)	NPA (TN/(TN+FP)) (95% CI)
	ANT	13	100% (1/1) (20.65 - 100)	100% (12/12) (75.75 - 100)	100% (1/1) (20.65 - 100)	100% (12/12) (75.75 - 100)
	CTX-M-1	13	0% (0/1) (0 - 79.35)	100% (12/12) (75.75 - 100)	0% (0/1) (0 - 79.35)	100% (12/12) (75.75 - 100)
	CTX-M-2	13	-	100% (13/13) (77.19 - 100)	-	100% (13/13) (77.19 - 100)
	P. aeruginosa gyrA [c]	12 [d]	100% (7/7) (64.57 - 100)	100% (5/5) (56.55 - 100)	85.7% (6/7) (48.69 - 97.43)	100% (5/5) (56.55 - 100)
	KPC	13	-	100% (13/13) (77.19 - 100)	-	100% (13/13) (77.19 - 100)
	NDM	13	-	100% (13/13) (77.19 - 100)	-	100% (13/13) (77.19 - 100)
	OXA-1	13	50% (1/2) (9.45 - 90.55)	100% (11/11) (74.12 - 100)	50% (1/2) (9.45 - 90.55)	100% (11/11) (74.12 - 100)
	PER	13	-	100% (13/13) (77.19 - 100)	-	100% (13/13) (77.19 - 100)
	SHV	13	-	100% (13/13) (77.19 - 100)	-	100% (13/13) (77.19 - 100)
	TEM	13	0% (0/1) (0 - 79.35)	100% (12/12) (75.75 - 100)	0% (0/1) (0 - 79.35)	100% (12/12) (75.75 - 100)
	VEB [d]	13	-	92.3% (12/13) (66.69 - 98.63)	-	92.3% (12/13) (66.69 - 98.63)
	VIM	13	100% (4/4) (51.01 - 100)	100% (9/9) (70.08 - 100)	100% (4/4) (51.01 - 100)	100% (9/9) (70.08 - 100)
P. mirabilis	AAC [e, f]	13	80% (4/5) (37.55 - 96.38)	75% (6/8) (40.93 - 92.85)	100% (5/5) (56.55 - 100)	100% (8/8) (67.56 - 100)
	ANT	13	100% (1/1) (20.65 - 100)	100% (12/12) (75.75 - 100)	100% (1/1) (20.65 - 100)	100% (12/12) (75.75 - 100)
	APH	13	100% (2/2) (34.24 - 100)	100% (11/11) (74.12 - 100)	100% (2/2) (34.24 - 100)	100% (11/11) (74.12 - 100)
	armA [g]	13	100% (1/1) (20.65 - 100)	91.7% (11/12) (64.61 - 98.51)	100% (1/1) (20.65 - 100)	91.7% (11/12) (64.61 - 98.51)
	CMY	13	100% (2/2) (34.24 - 100)	100% (11/11) (74.12 - 100)	100% (2/2) (34.24 - 100)	100% (11/11) (74.12 - 100)
	CTX-M-1 [h]	13	-	92.3% (12/13) (66.69 - 98.63)	-	100% (13/13) (77.19 - 100)

Organism	Resistance Marker	Number of Testing Events	Blood Agar		MacConkey Agar	
			PPA (TP/(TP+FN)) (95% CI)	NPA (TN/(TN+FP)) (95% CI)	PPA (TP/(TP+FN)) (95% CI)	NPA (TN/(TN+FP)) (95% CI)
	CTX-M-2 [f, i]	13	0% (0/1) (0 - 79.35)	91.7% (11/12) (64.61 - 98.51)	100% (1/1) (20.65 - 100)	100% (12/12) (75.75 - 100)
	CTX-M-9	13	100% (2/2) (34.24 - 100)	100% (11/11) (74.12 - 100)	100% (2/2) (34.24 - 100)	100% (11/11) (74.12 - 100)
	DFR	13	100% (2/2) (34.24 - 100)	100% (11/11) (74.12 - 100)	100% (2/2) (34.24 - 100)	100% (11/11) (74.12 - 100)
	KPC	13	-	100% (13/13) (77.19 - 100)	-	100% (13/13) (77.19 - 100)
	NDM	13	100% (1/1) (20.65 - 100)	100% (12/12) (75.75 - 100)	100% (1/1) (20.65 - 100)	100% (12/12) (75.75 - 100)
	OXA-1 [h]	13	100% (2/2) (34.24 - 100)	90.9% (10/11) (62.26 - 98.38)	100% (2/2) (34.24 - 100)	100% (11/11) (74.12 - 100)
	OXA-9 [f]	13	0% (0/1) (0 - 79.35)	100% (12/12) (75.75 - 100)	100% (1/1) (20.65 - 100)	100% (12/12) (75.75 - 100)
	OXA-48 [h]	13	-	92.3% (12/13) (66.69 - 98.63)	-	100% (13/13) (77.19 - 100)
	Sul2 [f]	13	83.3% (5/6) (43.65 - 96.99)	100% (7/7) (64.57 - 100)	100% (6/6) (60.97 - 100)	100% (7/7) (64.57 - 100)
	TEM [f]	13	83.3% (5/6) (43.65 - 96.99)	100% (7/7) (64.57 - 100)	100% (6/6) (60.97 - 100)	100% (7/7) (64.57 - 100)
	VEB	13	-	100% (13/13) (77.19 - 100)	-	100% (13/13) (77.19 - 100)
	VIM	13	-	100% (13/13) (77.19 - 100)	-	100% (13/13) (77.19 - 100)

[a] False negatives were obtained with one isolate on MacConkey agar for AAD and Sul1. This isolate was repeated on both media, and true positives were obtained for AAD and Sul1 on both media. Substituting original results with retest results would improve isolate (testing event) agreement across all AMR genes to 100% for *K. pneumoniae* on MacConkey media.
[b] False negatives were obtained with one isolate on blood agar for CTX-M-1, RMT and TEM. Repeat testing of this isolate on both media produced false negatives for CTX-M-1, RMT and TEM on both media, which is not consistent with a media effect. Adjudication testing was performed with this isolate in duplicate on both agar media and true positives for CTX-M-1, RMT and TEM were obtained for all samples. Substituting original results with these adjudication test results would improve isolate (testing event) agreement across all AMR genes to 100% for *K. pneumoniae* on blood media.
[c] A false negative was obtained with one isolate on MacConkey agar for *P. aeruginosa gyr*A mutant. This isolate was repeated on both media, and true positives were obtained for *P. aeruginosa* gyrA mutant on both media. Substituting original results with retest results would improve isolate (testing event) agreement across all AMR genes to 100% for *P. aeruginosa* on both media.
[d] One isolate was not evaluated for *P. aeruginosa* gyrA due to a mismatch in species ID from WGS and the expected species. *E. coli* was reported by whole genome sequencing, but the isolate was expected to be *P. aeruginosa*. Results of re-sequencing for this isolate agree with the Acuitas AMR Gene panel results for the isolate producing true positives for *P. aeruginosa* gyrase mutant and VEB with true negatives for all other AMR gene variants on both media.
[e] False positives were obtained with two isolates on blood agar for AAC. The two isolates were repeated on both media, and true negatives were obtained for AAC for both isolates on both media.
[f] False negatives were obtained with one isolate on blood agar for AAC, CTX-M-2, OXA-9, Sul2 and TEM. This isolate was repeated on both media, and true positives were obtained for AAC, CTX-M-2, OXA-9, Sul2 and TEM on both media.

[g] False positives were obtained with one isolate on both media for armA. Substituting original results with retest results would improve isolate (testing event) agreement across all AMR genes to 92% (12/13) for *P. mirabilis* on both media. The lack of 100% agreement is due to the false positives on both media for armA, which is not consistent with a media effect.
[h] False positives were obtained with one isolate on blood agar for CTX-M-1, OXA-1 and OXA-48. This isolate was repeated on both media, and true negatives were obtained for CTX-M-1, OXA-1 and OXA-48 on both media.
[i] A false positive was obtained with one isolate on blood agar for CTX-M-2. This isolate was repeated on both media, and true negatives were obtained for CTX-M-2 on both media.

9. Carry-over/Cross-Contamination

Carry-over/cross contamination between samples tested by the Acuitas AMR Gene Panel was evaluated. A panel of twelve (12) isolates representing organisms targeted by the Acuitas AMR Gene Panel test, harboring a variety of the targeted resistance genes, and a negative control isolate were subject to testing; refer to *Table 22 - Carry-over/Cross-Contamination Test Panel*.

Table 22 - Carry-over/Cross-Contamination Test Panel

Parent LDW Number/ External ID	Study LDW Number	Organism	AMR Genes Detected by Whole Genome Sequencing	Operator
L00008802-001	L00021588-001	*E. coli*	AAC, *E. coli gyr*A Mutant, OXA-1, SHV, Sul1	1
L00009106-001	L00021589-001	*E. coli*	AAC, Sul1, TEM	1
L00000411-001	L00021590-001	*K. pneumoniae*	AAC, AAD, APH, KPC, Sul1, TEM	1
L00011693-001	L00021591-001	*P. aeruginosa*	AAC, ANT, CTX-M-2, *P. aeruginosa gyr*A Mutant	1
L00012604-001	L00021592-001	*P. mirabilis*	AAC, APH, CTX-M-9, DFR, OXA-1, Sul2, TEM	1
L00014668-001	L00021593-001	*E. faecalis*	None	1
L00008788-001	L00021594-001	*E. coli*	AAC, CMY, *E. coli gyr*A Mutant, SHV, Sul1, Sul2, TEM	2
L00009581-001	L00021595-001	*K. pneumoniae*	AAC, AAD, CTX-M-1, DFR, OXA-9, Sul1, Sul2, TEM	2
L00007838-001	L00021596-001	*K. pneumoniae*	AAC, AAD, CTX-M-1, NDM, RMT, Sul2, TEM	2
L00007586-001	L00021597-001	*P. aeruginosa*	*P. aeruginosa gyr*A Mutant, VIM	2
L00000276-001	L00021598-001	*P. mirabilis*	AAC, armA, CTX-M-1, Sul2, TEM	2
L00021587-001	L00021599-001	*E. faecalis*	None	2

Two (2) operators prepared and blinded a panel of six (6) positive isolates and six (6) negative control isolates (a *S. aureus* isolate negative for all resistance markers) for testing by the other operator. Isolates were cultured on blood agar, 0.5 McFarland suspensions were prepared, and DNA was extracted. Positive and negative control samples were tested in an alternating fashion on the Acuitas AMR Gene Panel assay plates. There was 100% agreement between observed and expected positive/negative results (*Table 23 - Carry-over/Cross-Contamination Results)* without evidence of carry-over/cross-contamination between samples tested by the Acuitas AMR Gene Panel.

Table 23 - Carry-over/Cross-Contamination Results [a]

Sample Number For Study	# Results	# Positive Results/# Expected Positive Results (%)	# Negative Results/# Expected Negative Results (%)
L00021588-001	17	6/6 (100%)	11/11 (100%)
L00021589-001	17	4/4 (100%)	13/13 (100%)
L00021590-001	18	6/6 (100%)	12/12 (100%)
L00021591-001	14	5/5 (100%)	9/9 (100%)
L00021592-001	18	7/7 (100%)	11/11 (100%)
L00021593-001	1	0/0 (-)	1/1 (100%)
L00021594-001	17	8/8 (100%)	9/9 (100%)
L00021595-001	18	8/8 (100%)	10/10 (100%)
L00021596-001	18	7/7 (100%)	11/11 (100%)
L00021597-001	14	3/3 (100%)	11/11 (100%)
L00021598-001	18	5/5 (100%)	13/13 (100%)
L00021599-001	1	0/0 (-)	1/1 (100%)
CP3416 – Negative Control (NC)	420	0/0 (-)	420/420 (100%)

[a.] Table includes reported AMR gene results per organism as described in Table 1 along with species ID results for *E. coli* and *P. aeruginosa* as used in conjunction with mutant gyrase results for these two organisms.

B. CLINICAL PERFORMANCE

1. Introduction

The performance characteristics of the Acuitas AMR Gene Panel with bacterial isolates were determined in a multi-site investigational clinical study by comparing the Acuitas AMR Gene Panel with Whole Genome Sequencing (WGS), species identification by MALDI-TOF MS, and Antimicrobial Susceptibility Testing (AST) by broth microdilution. Four geographically diverse sites participated in the testing of isolates either prospectively collected or stocked and de-identified for use in the Clinical Performance Evaluation.

Isolates included in the study had been previously identified as Enterobacterales*, Pseudomonas aeruginosa,* or *Enterococcus faecalis*. Study samples included bacterial isolates grown on blood agar.

The Clinical Performance Evaluation was performed between September 11, 2018 and May 2, 2019 with final results reported for a total of 1,307 isolate samples (1,224 clinical stock isolate samples and 83 prospective isolate samples).

- Isolates harboring rare resistance genes were replicated with a goal of achieving 50 unique testing events per gene target.
- Each of the 83 prospective isolate samples were unique and enrolled at clinical sites. Isolates enrolled in the prospective arm of the clinical performance evaluation were selected based on their identification (enrolling when organisms are one of the bacterial species detected by the test: Enterobacterales, *Pseudomonas aeruginosa,* and *Enterococcus faecalis)* and documented non-susceptibility, excluding intrinsic resistance, to at least one (1) of the

following antibiotic classes, where appropriate: Aminoglycosides, Carbapenems, Cephalosporins (including β-Lactam Combination Agents), Fluoroquinolones, Penicillins, Polymyxins, Sulfonamides, Trimethoprim, or Vancomycin.

For testing with the Acuitas AMR Gene Panel, well-isolated colonies that grew on blood agar were diluted to a 0.5 McFarland standard equivalent suspension using the direct colony suspension method per CLSI M07[1] Approved Standard.

2. Reference Methods

a) Culture ID/AST

Organism identification was confirmed for the Gram-negative organisms (e.g., Enterobacterales and *Pseudomonas aeruginosa*) isolates in the clinical study using an automated species identification method. Testing by broth microdilution was used to determine Antimicrobial Susceptibility for a given Gram-negative isolate according to which CLSI M100[2] 28th edition breakpoints and FDA-specified exceptions for the SDO recommended breakpoints available on the FDA's Antibacterial Susceptibility Test Interpretive Criteria[3] website were applied.

Organism identification for Gram-positive organisms (e.g., *Enterococcus faecalis*) was performed via MALDI-TOF MS and antimicrobial susceptibility testing by broth microdilution.

b) Whole Genome Sequencing

Detection of AMR genes in the clinical study isolates by the Acuitas AMR Gene Panel was confirmed against WGS results for each isolate using a validated WGS pipeline using the Illumina Hi-Seq 4000 platform. A glycerol stock of each bacterial isolate that was assessed by the Acuitas AMR Gene Panel was sent for sequencing for comparison.

3. Results

Results and performance of the Acuitas AMR Gene Panel for detection of AMR genes versus WGS results in the Clinical Evaluation are detailed in *Table 24 - Clinical Performance for the Acuitas AMR Gene Panel (PCR/WGS)* which summarizes the Acuitas AMR Gene Panel assays for applicable organisms according to *Table 1 - Antimicrobial Resistance Gene Markers Associated with Bacterial Species.* Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) for AMR genes ranged from 94.4% to 100% and 96.5 to 100%, respectively.

'Total Unique Strains' in *Table 24 - Clinical Performance for the Acuitas AMR Gene Panel (PCR/WGS)* is the number of unique strains tested per AMR Gene Target, limited to species for which the AMR gene is reported as indicated in *Table 24.* A subset of 'Total Unique Strains' were tested in replicate as indicated by 'Total Replicates' in *Table 26*. WGS results for the unique strains and replicates are indicated in the last four columns of *Table 26.*

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[1] Clinical Laboratory Standards Institute (CLSI). *M07 Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically; 11th Edition*. Wayne, PA; 2018.
[2] Clinical Laboratory Standards Institute (CLSI). *M100 Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Eighth Informational Supplement*. Wayne, PA; 2018.
[3] U.S. Food and Drug Administration (2020). *Antibacterial Susceptibility Test Interpretive Criteria*. Available from: https://www.fda.gov/drugs/development-resources/antibacterial-susceptibility-test-interpretive-criteria

Table 24 - Clinical Performance for the Acuitas AMR Gene Panel (PCR/WGS)

AMR Gene Target	PPA			NPA			Total Unique Strains (n)	Total Replicates (n)[r]	Positive for AMR Targets by WGS		Negative for AMR Target by WGS	
	TP/ (TP+FN)	%	95% CI	TN/ (TN+FP)	%	95% CI			Unique Strains (n)	Replicates (n)[r]	Unique Strains (n)	Replicates (n)[r]
AAC	610/(622)[a-c]	98.1	96.66-98.89	536/(545)[d-e]	98.3	96.89-99.13	577	732	315	386	262	346
AAD	128/(130)	98.5	94.56-99.58	192/(199)[f]	96.5	92.92-98.29	185	189	98	54	87	135
ANT	203/(205)	99.0	96.51 - 99.73	628/(633)[g]	99.2	98.16 - 99.66	392	543	64	168	328	375
APH	39/(40)	97.5	87.12-99.56	443/(444)	99.8	98.74-99.96	263	280	31	12	232	268
armA	8/(8)	100.0	67.56-100.00	147/(147)	100.0	97.45-100.00	78	91	4	8	74	83
CMY	126/(128)[h]	98.4	94.48-99.57	688/(691)[i]	99.6	98.73-99.85	422	489	55	84	367	405
CTX-M-1	264/(273)	96.7	93.85-98.26	929/(938)[j]	99.0	98.19-99.49	621	732	162	143	459	589
CTX-M-2	35/(35)	100.0	90.11-100.00	801/(803)	99.8	99.10-99.93	392	543	21	27	371	516
CTX-M-9	73/(74)	98.6	92.73-99.76	781/(782)	99.9	99.28-99.98	459	489	58	23	401	466
DFR	167/(169)	98.8	95.79-99.67	646/(650)	99.4	98.43-99.76	422	489	90	96	332	393
DHA	36/(36)	100.0	90.36-100.00	293/(293)	100.0	98.71-100.00	185	189	33	6	152	183
***E. coli gyr*A Mutant**	160/(163)	98.2	94.73-99.37	167/(168)	99.4	96.71-99.89	155	209	81	101	74	108
IMP	72/(72)	100.0	94.93-100.00	257/(257)	100.0	98.53-100.00	185	189	5	71	180	118
KPC	75/(77)	97.4	91.02-99.28	1130/(1134)	99.6	99.10-99.86	621	732	63	21	558	711
MCR-1	51/(54)[k]	94.4	84.89-98.09	281/(281)	100.0	98.65-100.00	159	209	14	48	145	161
NDM	56/(57)	98.2	90.71-99.69	801/(805)[l]	99.5	98.73-99.81	448	523	47	17	401	506
OXA-1	240/(249)	96.4	93.27 - 98.09	910/(918)	99.1	98.29 - 99.56	577	732	112	161	465	571
OXA-9	58/(58)	100.0	93.79-100.00	760/(761)	99.9	99.26-99.98	422	489	47	21	375	468
OXA-48	59/(62)	95.2	86.71-98.34	448/(452)	99.1	97.75-99.66	293	280	48	27	245	253
PER	81/(82)	98.8	93.41-99.78	265/(266)	99.6	97.90-99.93	155	243	9	81	146	162
***P. aeruginosa gyr*A Mutant**	265/(279)[m]	95.0	91.75-96.99	67/(68)	98.5	92.13-99.74	154	243	103	216	51	27
RMT	31/(32)	96.9	84.26-99.45	297/(297)	100.0	98.72-100.00	185	189	27	10	158	179
SHV	12/(12)	100	75.75 – 100.00	668/(671)	99.6	98.69 - 99.85	314	452	10	4	304	448
Sul1	420/(424)	99.1	97.60-99.63	232/(240)[n]	96.7	93.56-98.30	344	398	226	249	118	149
Sul2	489/(501)	97.6	95.86-98.62	307/(318)[o]	96.5	93.91-98.06	422	489	212	331	210	158
TEM	600/(609)[p]	98.5	97.22 - 99.22	559/(572)[q]	97.7	96.15 - 98.67	591	732	277	391	314	341
vanA	57/(57)	100.0	93.69-100.00	36/(36)	100.0	90.36-100.00	43	54	8	52	35	2
VEB	89/(89)	100.0	95.86-100.00	411/(414)	99.3	97.89-99.75	233	334	24	72	209	262
VIM	91/(93)	97.8	92.49-99.41	409/(410)	99.8	98.63-99.96	233	334	22	80	211	254

[a] One (1) FN result attributed to the presence of an aac(3)-IIa gene variant that had no valid alignment with the primers/probe of the AAC assay harbored by a single K. pneumoniae unique isolate.

[b] *Two (2) FN results due to testing of two (2) replicates of a single unique E. coli isolate.*
[c] *Two (2) FN results due to testing of two (2) replicates of a single unique P. aeruginosa isolate.*
[d] *One (1) FP result attributed to the presence of a truncated aac(3)-II gene harbored by a single unique E. coli isolate.*
[e] *One (1) FP result attributed to the presence of a truncated aac(3)-Ib gene in a single unique K. pneumoniae isolate.*
[f] *Three (3) FP results attributed to an aadA15 gene variant harbored by three (3) K. pneumoniae isolates with high numbers (≥3) of mismatches in the reverse primer of the AAD assay, with two (2) isolates tested as replicates of a single unique strain. Two (2) additional FP results from K. pneumoniae isolates demonstrated alignment of the AAD assay primers/probe with high numbers of mismatches (≥3) in the reverse primer, but no attributable gene variant was detected in the AR database used for analysis.*
[g] *One (1) FP result attributed to high PCR baseline drift and not true amplification of the ANT target assay in one (1) unique P. aeruginosa isolate.*
[h] *Two (2) FN results attributed to the presence of CMY gene variants with high numbers of mismatches (≥3) to the primers of the CMY assay in two (2) E. coli isolates. One (1) isolate harbored a blaCMY-2 gene variant and one (1) isolate harbored a blaCMY-42 gene variant.*
[i] *Four (4) FP results attributed to the presence of a CMY gene variant with high numbers of mismatches (≥3) to the primers of the CMY assay in four (4) E. coli isolates. These four (4) E. coli isolates represented two (2) unique strains, each tested in two (2) replicates. Both isolates harbored a blaCMY-4 gene variant. Seven (7) FP results attributed to the presence of a blaCMY-16 gene variant with high numbers of mismatches (≥3) to the reverse primer of the CMY assay in six (6) unique K. pneumoniae isolates, with one isolate tested in two (2) replicates. One (1) FP result attributed to the presence of a blaCMY-16 gene variant with high numbers of mismatches (≥3) to the reverse primer of the CMY assay in one (1) P. mirabilis isolate.*
[j] *Seven (7) FP results attributed to the presence of a blaCTX-M-27 gene variant with perfect alignment to the primers/probe of the CTX-M-1 assay for 7 replicates of 1 unique E. coli isolate that was not originally identified by WGS analysis.*
[k] *Three (3) FN results due to testing of three (3) replicates of a single E. coli isolate.*
[l] *Four (4) FP results due to testing of four (4) replicates of a single K. pneumoniae isolate.*
[m] *One (1) FN result attributed to a negative result for the P. aeruginosa ID assay for a single unique P. aeruginosa isolate. Amplification of the P. aeruginosa gyrA Mutant assay was present for this isolate.*
[n] *Two (2) FP results due to testing of two (2) replicates of one unique E. coli isolate.*
[o] *Four (4) FP results due to testing for four (4) replicates of a single unique K. pneumoniae isolate.*
[p] *Two (2) FN results due to testing of two (2) replicates of a single unique E. coli isolate.*
[q] *Four (4) FP results due to testing for four (4) replicates of a single unique K. pneumoniae isolate.*
[r] *Replicates are the total number of samples for unique isolates tested multiple times. For example, replicates would equal 5 if three unique isolates were respectively tested in singlicate, duplicate and triplicate.*

Please refer to the Acuitas AMR Gene Panel Electronic User Guide (EUG) at www.opgen.com for further complementary performance information as well as details on antibiotic drug classes and for organism/drug/resistance marker combinations for which association with resistance was demonstrated.

III. CONCLUSIONS

The results of the analytical and clinical performance studies summarized above demonstrate that the Acuitas AMR Gene Panel is safe and effective for its intended use and is substantially equivalent to the predicate device.